HISTOPATHOLOGICAL ANALYSIS OF SALIVARY GLAND LESIONS AND ROLE OF IMMUNOHISTOCHEMISTRY IN DIFFERENTIAL DIAGNOSIS

DISSERTATION

SUBMITTED FOR M.D. (PATHOLOGY)

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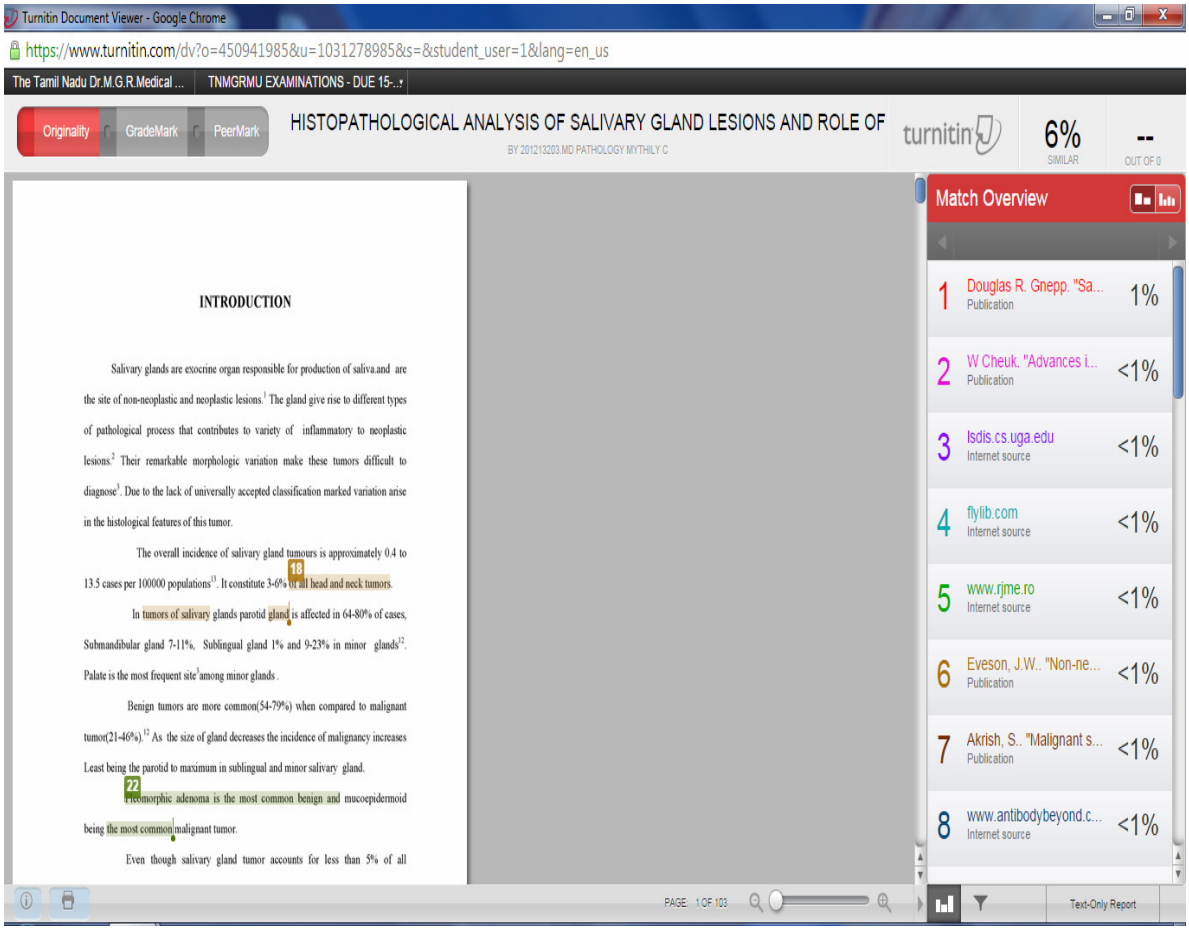
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INTRODUCTION
Salivary glands are exocrine organ responsible for production of saliva.and are the site of non-neoplastic and neoplastic lesions.[1] The gland give rise to different types of pathological process that contributes to variety of inflammatory to neoplastic lesions.[2] Their remarkable morphologic variation make these tumors difficult to diagnose[3]. Due to the lack of universally accepted classification marked variation arise in the histological features of this tumor.
The overall incidence of salivary gland tumours is approximately 0.4 to 13.5 cases per 100000 populations[13]. It constitute 3-6% of all head and neck tumors.
In tumors of salivary glands parotid gland is affected in 64-80% of cases, Submandibular gland 7-11%, Sublingual gland 1% and 9-23% in minor glands[32]. Palate is the most frequent site[3] among minor glands .
Benign tumors are more common(54-79%) when compared to malignant tumor(21-46%).[12] As the size of gland decreases the incidence of malignancy increases Least being the parotid to maximum in sublingual and minor salivary gland.
Pleomorphic adenoma is the most common benign and mucoepidermoid being the most common malignant tumor.
Even though salivary gland tumor accounts for less than 5% of all
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CONTENTS

ABSTRACT

BACKGROUND:

Tumors of salivary gland have diverse histological forms and unpredictable clinical behavior. The diverse site of origin and complexity of classification further compound the difficulties in diagnosis. The main application of immunohistochemistry in salivary gland tumors is to demonstrate the existence of myoepithelial/basal cells or luminal cells.

OBJECTIVES:

1. To determine the incidence, age, sex, site distribution of lesions in various salivary glands and to study the histomorphological appearance of these lesions. 2. To study the expression of various Immunohistochemical markers in salivary gland tumors.

METHODS:

Surgically resected specimens received at Department of pathology, Thanjavur medical college were subjected to histopathological examination. Specimen were fixed in 10% formalin, processed and embedded in paraffin blocks. Serially cut to get sections of 3-5microns thickness, stained with Hemotoxylin and Eosin, Histochemistry and Immunohistochemistry were done wherever necessary.

RESULTS:

The total number of specimens was 92, of which 41were neoplastic (benign44.56% and malignant 25%) 28 were nonneoplastic. Pleomorphic adenoma was the commonest benign tumor accounting for73.17% of benign tumors. Mucoepidermoid carcinoma was the most common malignant tumor accounting for 52.18% of malignant tumors. Parotid is the common salivary gland involved followed by minor salivary and submandibular gland.salivary gland tumors shows slight female preponderance with male female ratio of 1:1.7. Expression of p63 in pleomorphic adenoma has confirmed the role of myoepithelial cells in the histiogenesis of this tumor. Lack or minimal expression of p63 in Mucoepidermoid indicates minimal myoepithelial cell differentiation in these tumors.

CONCLUSION:

Histopathology is still the goldern standard for diagnosis of salivary gland tumor. IHC do not directly indicate a definite diagnosis. It can enhance the accuracy and be a helpful tool when the diagnosis cannot be assessed by histological examination such as cell of origin, cell proliferation and tumor protein expression.

Keywords: Pleomophic adenoma, Mucoepidermoid carcinoma, Immunohistochemistry.

INTRODUCTION

Salivary glands are exocrine organ responsible for production of saliva and are the site of non-neoplastic and neoplastic lesions.[1] The gland give rise to different types of pathological process that contributes to variety of inflammatory to neoplastic lesions.[2] Their remarkable morphologic variation make these tumors difficult to diagnose[3]. Due to the lack of universally accepted classification marked variation arise in the histological features of this tumor.

The overall incidence of salivary gland tumours is approximately 0.4 to 13.5 cases per 100000 populations.[13] It constitute 3-6% of all head and neck tumors.

In tumors of salivary glands parotid gland is affected in 64-80% of cases, Submandibular gland 7-11%, Sublingual gland 1% and 9-23% in minor glands[12]. Palate is the most frequent site[3]among minor glands .

Benign tumors are more common(54-79%) when compared to malignant tumor(21-46%).[12] As the size of gland decreases the incidence of malignancy increases Least being the parotid to maximum in sublingual and minor salivary gland.

Pleomorphic adenoma is the most common benign and mucoepidermoid being the most common malignant tumor.

Even though salivary gland tumor accounts for less than 5% of all neoplasm, it is important because almost all lesions whether benign or malignant will present as swelling of parotid gland.

The prognosis is less favorable in parotid gland, most favourable in palate, and least favorable in submandibular gland with respect to diagnosis, treatment and prognosis[4].

Immunohistochemistry enhances the accuracy of diagnosis of cases that cannot be assessed by histological examination.Inspite of this Hematoxylin - Eosin staining is the golden method of diagnosis. Immunohistochemistry detects the nature of cell differentiation, cell proliferation and tumor protein expression[5].

Main application of immunohistochemistry in salivary gland tumor is to demonstrate the existence of luminal or myoepithelial/basal component when diagnosis is uncertain. Using these particular luminal myoepithelial markers salivary gland tumor divided into two groups

1.Tumors with dual cellular population .

2.Tumor with single line of diffentiation.[19]

AIM OF THE STUDY

1. To study incidence of salivary gland lesions.
2. To study the age, sex,and site distribution of various salivary gland lesions.
3. To study the histomorphological appearance of salivary gland lesions.
4. To evaluate myoepithelial marker p63 in Pleomorphic Adenoma and Mucoepidermoid carcinoma of salivary glands for differential diagnosis of these tumors and specification of their histiogenesis.
5. To study the expression of various Immunohistochemical markers in salivary gland tumors.

MATERIALS AND METHODS

Patients presenting with signs and symptoms of salivary gland enlargement in Thanjavur medical college hospital, Thanjavur during the period from September 2011- August2014 were included in this study, irrespective of the age group and sex.

A proforma [Annexure II] was made and all the patients attending the hospital with complaints related to salivary glands were made to fill the proforma.

All the surgical specimens received in the department of pathology were fixed in 10% neutral buffered formalin.

Grossing of the specimens were done with atmost care, noting the size of the lesion, whether they have circumscribed or infiltrative borders and presence of cystic changes were noted with special attention to the number of cysts, single or multiple, appearance of the surface, color of the walls, presence of papillary projections into the lumen of the cyst wall. All the suspicious were grossly sectioned and subjected to histopathological examination.

Sections were processed as small sections of 2-3mm in thickness in the automatic tissue processor and processed in a routine way.

Sections of 5µ thickness were cut and stained with Hematoxylin and Eosin and in doubtful cases slides were submitted for special histochemical stains such as PAS, Alcian blue, Masson trichrome were done[Annexure IV]. Histological classifications of these tumors were done as per WHO classification[2005] [Annexure III].

Immunohistochemistry of CK, p63, CD117, Her2/neu, Ki67 was done on deparaffinized 5µ sections after antigen retrieval by heat using microwave oven [Annexure V].

The intensity of Immunostaining reaction has been described from literature data[122] as follows.

- When the immunostaining reaction is all over or intensely positive,obvious even in small magnification[+++]
- when the immunostaining reaction is focal or with moderate intensity,evident on average magnification.[++]
- When the immunostaining reaction is weak or very focal,visible only at strong magnification[+]
- When the immunostaining reaction is very reduced,to limit[+/-].
- when the immunostaining is negative.[-]

Ki-67: The most stained areas in each section were chosen, and the minimum of thousand tumor cells were counted under light microscope fields [X400]. The percentage of positive tumor cell nuclei was recorded as labeling index[LI].

REVIEW OF LITERATURE

Salivary glands are tubulo acinar exocrine organs responsible for production and excretion of saliva

Anatomy.[9,10]

Salivary gland comprises three paired major glands. Largest being parotid, followed by submandibular and sublingual gland.

There are also several hundred minor glands which are distributed throughout oral and oropharyngeal submucosa in the upper respiratory and sinonasal tract.

Secretory acinus, related ducts and myoepithelial cells are the functional unit of salivary gland. Acinus may be mucous, serous or mixed.

Parotid is the largest of all salivary gland and it weighs about 14-30gms.

It is surrounded by thin capsule. Facial nerve divide it into two portions.

Superficial lobe is flattened and quadrilateral in shape. Majority of salivary gland tumors arise here.

Deep lobe is irregular, wedge shaped and is in anatomical relationship with parapharyngeal space. Excretory duct of parotid is Stenson's duct.The duct opens into parotid papillae opposite 2nd molar tooth.

SUBMANDIBULAR:

It is encapsulated and lies inside the submandibular triangle and weighs 7-8gms. It is organized into lobules and connected to main excretory duct(Wharthin's duct).Wharthin's duct opens into a narrow orifice called caruncula sublingualis.

SUBLINGUAL GLAND:

It weighs 3gms and lies in sublingual fossa and surrounded by loose connective tissue. It opens through a main duct called Bartholin's duct, which opens into submandibular duct and various small ducts(Rivinus ducts) which joins the submandibular duct and opens separately into mouth in plica sublingualis.

MINOR SALIVARY GLANDS:

More glands are present at the junction of soft and hard palate, lip, buccal mucosa, tongue, floor of mouth retromolar pad lateral aspect of tongue. In addition minor glands are present in ventral surface of tongue(Glands of Blandin and Nuhn)

Minor glands opens into the groove surrounding the circumvallate papillae(von Ebner's glands) are purely serous in type. Minor salivary glands are purely serous in type.Glands are not encapsulated and glands in the tongue are deeply located in the underlying muscle.

HISTOLOGY OF SALIVARY GLANDS:

The secretory acinus and the related ducts and myoepithelial cells forms the functional unit of salivary gland.

They are 3 types of secretory units serous, mucous or mixed

Serous unit contain amylase, mucinous, unit contain sialomucin and mixed unit made up of serous and mucous cells. Parotid is of serous type. Palatal salivary glands are predominantly mucous.

Submandibular and sublingual are of mixed type. These mixed unit are characterized by crescent shaped caps the demilunes of serous cells at the end of mucous secreting tubules.

ACINI:

The epithelial cells are pear shaped in serous acini , surrounded by a distinct basement membrane. The nucleus are basally situated and dense cytoplasm packed with **PAS positive zymogen granules**.

Mucinous acini are irregular in shape with clear cytoplasm filled with vacuoles of sialomucin(Alcian blue and mucicarmine +ve) and neutral sialomucin(PAS +ve)

DUCTS:

The intercalated and striated ducts are intra lobular and are called as secretory duct. Excretory ducts are interlobular.

Intercalated ducts are lined by single layer of cuboidal epithelium and an irregular myoepithelial cells. The striated ducts are lined by simple columnar epithelium.The principle duct consist of thick external fibrous coat of collagen and elastic fibres. Near the opening of mucous membrane the epithelium is stratified squamous.

BASKET CELLS OR MYOEPITHELIAL:

These are located between basement membrane and basal plasma membrane of acinar cells and are contractile in nature. There is only one myoepithelial cell for each secretory unit.

EMBRYOLOGY

Salivary gland development begins in 5^{th}-6^{th} week of age, when parotid gland primordial appears. Submandibular primordia develop at 6^{th} week, sublingual primordial at 7^{th} to 8^{th} week and intra oral minor salivary glands at 3^{rd} month of gestation.

Salivary gland arise as buds of proliferative epithelium from the primitive stomodeum(oral cavity).The stomodeum epithelium is partly endoderm and ectoderm. Oral ectoderm give rise to parotid gland where as endoderm give rise to submandibular and sublingual gland. Initially the epithelial cords and bulbs are without lumen. Luminization occurs 1^{st} in the epithelial cord and progress to terminal bulbs. Cellular differentiation results in the characteristic features of excretory ducts and acini.

Proximal branched cords become excretory and main ducts. Distal branched cords become striated ducts and the luminized terminal bulbs become intercalated ducts and acini.

The parotid gland is colonized by lymphocytes at 3^{rd} month of gestation. These subsequently develop into several intraparotid and periparotid lymphnodes. These nodules lack the complete organization of lymph node.

DEVELOPMENTAL DISORDERS:

During the normal course of human development aberration of salivary glands can occur.

HETEROTOPIC SALIVARY GLAND:

Heterotropia is a supernumerary normal structure in an abnormal location.

Salivary gland has been described in variety of aberrant locations hypophysis, cerebropontine angle, middle ear,[16]mastoid, auditory canal, tongue, palatine tonsil, thyroglossal duct, mandible, thyroid, parathyroid capsules and sternoclavicular joint. These are more common in lymph node near parotid gland.

Salivary gland elements are seen in association with developmental lesions such as branchial cyst.

Shin C Kim etal[17] in 2000 reported a case of choristoma of the anterior chest wall.

ACCESSORY PAROTID GLAND:

The term "accessory gland" refers to lobules of parotid that are separated from the body of parotid and opens into Stenson's duct. Length of accessory parotid duct 0.5-3cms. Clinically lesions of accessory parotid tissue are present as masses in cheek.

ONCOCYTOSIS:

It was first described by **Schaffer** and later the term was applied by **Hamperl.** Oncocyte are cell with numerous number of cytoplasmic mitochondria. The proliferation of oncocyte without any pathological significance is called oncocytosis or oncocytic metaplasia. Majority of cases are found in the parotid gland[7.]. Histologically present as solitary foci of enlarged eosinophilic epithelial cells.

ADENOMATOID HYPERPLASIA OF MUCOUS GLANDS:

Synonym: Hamartomatous proliferation of minor salivary gland. Clinically present as mass in palate that is covered with bluish or normal mucosa but shows no ulceration or pain.

MICROSCOPIC FEATURES: Mucosa shows pseudoepitheliomatous hyperplasia with underlying connective tissue contains enlarged lobules of normal appearing mucous acini and ducts. Salivary tissue is described as hyperplastic when present in greater than normal amounts and crowded.

POLYCYSTIC(DYSGENETIC) DISEASE OF PAROTID:

It is developmental malformation of duct system usually affect both salivary glands. Commonly affects female offspring and present as recurrent painless swelling.

GROSS: Lobular, cut surface shows mottled yellow and ivory nodules with a fine spongy consistency.

MICROSCOPY: Architecture is maintained, lobules are markedly distended which impact a honey combed or lattice like appearance. Small residual island of glandular acini are present between the cyst which vary in size and lined by flattened cuboidal or columnar epithelium.[14] Occasionally apocrine cells are seen in the luminal border.

OBSTRUCTIVE DISORDERS:

Can occur in major or minor salivary glands

1. Occur as a result of stasis of salivary secretion in ducts.
2. Partial or complete block of excretory ducts.
3. Traumatic severance of salivary ducts.

Three common primary obstructive condition are

1)Mucocele, 2)Mucous retention cyst, 3)Sialolithiasis.

MUCOCELE[11,12.]

Most common non neoplastic lesion with prevalence of 1.4/1000. These are pools of saliva within a cystic cavity.

Edmund et al conducted study of 594 cases in which majority of mucocele occurs in lower lip. Seen in the age group 11-20 years. Peak incidence of mucous retention cyst occur in 7^{th}-8^{th} decade of life. Clinically mucocele manifest as painless soft and fluctuant, dome shaped mucous nodules which are 0.2-1cm. Large mucoceles of floor of the mouth are referred as ranula. If they extend into cervical tissues-plunging ranula.

MICROSCOPY: Mucous escape reaction with a pool of mucous in fibrous connective tissue, surrounded by inflammatory cells predominantly macrophages. These mucus are highlightened by mucicarmine stain.[11]

MUCOUS RETENTION CYST OR SIALOCYST: Less common than mucocele, develop as a result of cystic dilatation due to obstruction of major salivary gland. Average age at presentation is 50 years. Mucous retention cyst of parotid is known **as salivary gland cyst** and is true cyst lined by epithelium. Lesions are mostly unilocular.

MICROSCOPY: Epithelial lined fibrous tissue around the mucous pool. The epithelium may be cuboidal, columnar or stratified squamous. Lumen of cyst may be filled with an eosinophilic material that stains positive for mucin[18.]

SIALOLITHIASIS.[10,11,12.]

It denotes various calcified masses that are formed in ducts of salivary gland[24]. It occurs as a result of mineralization of debris that has accumulated in the lumen of duct. According to published data, salivary stones are localized in submandibular glands in 80-90% of sialolithiasis cases. They vary in shape ranging from 2mm-2cms[25.]

SIALADENITIS:[(10,11,12.)]

Inflammation of salivary gland due to mechanical, physical, microbial, and immunological factors. Radiation is an important physical cause of sialadenitis. Serous acini of parotid gland are radiosensitive. Acute inflammatory response occur initially followed by chronic sialadenitis and xerostomia[24.]

ACUTE SIALADENITIS:[(10,11,12)]

Infection occurs due to decreased salivation and patient present with a state of dehydration. Infants in tube feeding are at risk of neonatal sialadenitis due to staphylococcus aureus and pseudomonas aeruginosa[12]infection.

CHRONIC SIALADENITIS[10,11.]

It is a non granulomatous chronic inflammatory disorder of salivary glands due to physical, microbial or immunological factors.

KUTTNER TUMOR OR CHRONIC SCLEROSING SIALADENITIS: This was described by **Kuttner** in 1896. It is a benign inflammatory process occurring almost exclusively in submandibular gland of middle aged adults.

MICROSCOPY: Initial phase shows chronic inflammation, duct ectasia with periductal fibrosis followed by acinar atrophy, fibrosis ductal dilatation and sclerosis in late stage[139].

INFECTIOUS SIALADENITIS

TUBERCULOSIS[10,11] The most common infection of that affects salivary gland is Mycobacterial adenitis involving the periparotid and intra parotid lymph nodes followed by other salivary glands. Symptoms manifest only at late stage of disease.

MICROSCOPY: Shows necrotising granulomatous inflammation with caseous center surrounded by epitheloid cells, langhans and foreign body giant cells.

VIRAL SIALADENITIS[10,11.]

MUMPS: The most common cause of parotid swelling are viral agents. Peak age of incidence is 4-6yrs.

MICROSCOPY: Shows ductal dilatation, acinar cell vacuolization and dense lympho-plasmacytic infiltrates.

CYTOMEGALOVIRUS:(CMV) Most people are infected with cytomegalovirus at some point in their life. The age of incidence varies world wide. In developing countries most infection is acquired during childhood. CMV can be sexually transmitted and via breast milk, organ transplantation and rarely blood transfusion.

MICROSCOPY: Hallmark of CMV infection in salivary gland is an enlarged duct cells with characteristic intranuclear viral inclusion bodies-owl's eye appearance.

HIV ASSOCIATED LYMPHOEPITHELIAL CYST:

Nodular or diffuse enlargement of salivary gland occurring in patient with HIV infection is called as HIV associated LEC, HIV-salivary gland disease, benign lymphoepithelial lesion.

GROSS: Multiple cyst of variable diameter upto 5cm can be seen usually in the superficial lobes of parotid with preservation of gland architecture. .

MICROSCOPY: Shows poorly circumscribed multinodular areas composed of multicystic structures surrounded by dense lymphoid infiltrate with florid lymphoid follicular hyperplasia and lymphoepithelial lesion.

The cyst are lined predominantly by non keratinizing, stratified squamous epithelium and focally by cuboidal type cells permeated by monocytoid/marginal zone-B cells and also sparsely by T-cells present within the lymphoid infiltrate.

BENIGN CYSTS OF PAROTID[10,11.]

LYMPHOEPITHELIAL CYST(LEC):

1) These are uncommon, mostly arising from Neisse Nicholson rests of parotid gland.

   Average age of patient is 45yrs present as a painless mass. Cyst measures 6cm in diameter(average 2.5cms).

   GROSS: Predominantly unilocular cyst with yellow white caseous material.

   MICROSCOPY: Epithelial cyst wall contain dense lymphoid tissue with prominent germinal centre.Lining is usually stratified squamous but focal areas of respiratory or cuboidal epithelium can occur.

   Differential diagnosis: 1)Warthin's tumor, 2)True branchial cleft cyst. 3)Low grade MEC 4)Cystic LESA.

2) **SALIVARY DUCT CYST:**[10,11] salivary duct cyst or sialocyst arise in parotid gland. Occurs in 4th decade.

   GROSS: Cyst usually present as a unilocular painless swelling, size measures upto 3cms in diameter

MICROSCOPY: Luminal surface is covered by cuboidal or squamous epithelium with chronic inflammation of the collagenous wall.

BENIGN LYMPOEPITHELIAL LESION[10,11]

Godwin in 1952 proposed the name Benign lympoepithelial lesion to describe parotid lesion previously called Mikulicz disease. Berner and Bhaskar[10] suggested that Benign lympoepithelial lesion is neither a neoplasm nor a lesion in which epithelium plays an aggressive or dominant role. It is a primary lymphoid lesion that can involve the salivary gland.

Age: 1-86yrs

Females>Males. (average age 51yrs for female and 45yrs for male)

83% occur in parotid.

11% submandibular gland.

6% in minor salivary gland.

GROSS: 1-2cms in size, well circumscribed.

MICROSCOPY: Epithelium lined by multiloculated cystic space encased by lymphoid tissue, plasma cells and germinal centers.

SJOGREN'S SYNDROME:[10,11.]

First described in 19th century by Haldane. Mikulicz a few years later it was named after ophthalmologist Sjogren whose classic report of this appeared in 1993.

Sjogren's is a chronic lymphoproliferative disorder which may present as a primary or secondary disease. The histopathology substrate is characterized by presence of lymphocytic infiltrate in glandular and extra glandular site.

MICROSCOPY: Shows the presence of lymphocytic infiltrate in glandular and extra glandular site.

KIMURA'S DISEASE: Kimura's is an angiolymphoid proliferative disorder of soft tissue with eosinophilia and elevated IgE levels. According to Kung et al Kimura's disease was first described in China during 1937. This title came after Kimura et al who reported a similar case in 1948. It occur predominantly in young and middle aged. Mean age 27-40yrs. M>F. **Rosai et al**[22] were first to note that Kimura disease is different from angiolymphoid hyperplasia. It occur mainly around the ear with frequent involvement of salivary gland.

GROSS: The lesions are rubbery and irregular or nodular.

C/S: Grey to light brown and may contain embedded lymph nodes and attached salivary glands and muscle.

MICROSCOPY: Uncapsulated lesion characterized by fibro collagenous tissue, lymphoid tissue and mixed inflammatory cell infiltrate with numerous eosinophils. Eosinophilic abscess, polykaryocytes of Warthin- Finkeldey are sometimes present in germinal centres .

TUMOUR LIKE LESIONS:[10,11,12]

NECROTIZING SIALOMETAPLASIA:

Benign self limiting reactive inflammatory condition salivary gland.First reported in minor salivary gland of hard palate by **Abram's** et al in 1973. It clinically and histologically simulates MEC, SCC.

ETIOLOGY:

Ischemia caused by impairment of vascular supply to salivary gland tissue has been implicated as the most likely etiology for this disease. By both clinical and experimental evidence, lesions are associated with trauma, local anaesthetic injection, allergy.

EPIDEMIOLOGY:

Accounts for 0.3% of all biopsied lesion of oral cavity. Most cases occur in palate(77.2%). Others include minor salivary glands of lower lip, retromolar pad area, tongue , buccal mucosa and major salivary gland(8.7%).

Age: Average 45.9yrs: Sex: M:F 2:1.

CLINICAL FEATURES: Deep crater like ulcer of palate that resembles a malignant process. Lesions ranges from 0.5cm-5cms in size.

MICROSCOPY: Lobular infarction or necrosis of acini, squamous metaplasia of residual acini and ducts are predominant . Squamous cell nucleus appears bland. Lymphocytes and neutrophils infiltrates the surrounding tissue. Lobular architecture will be maintained despite extensive metaplastic and inflammatory changes.

DD: Squamous cell carcinoma, MEC, Coagulative necrosis of acini.

SCLEROSING POLYCYSTIC ADENOSIS: First reported by **Smith and colleagues** in 1996 from Armed Force Institute of pathology. Characterized by sclerosed, collagenous tissue surrounding ill defined acinar and ductal lobules with cystically dilated duct structures. Age: 9-75yrs with Male:Female ratio of 14:22.

Site: Most often occur in parotid gland.

GROSS: Lesions are well circumscribed, multifocal rubbery nodules with size ranging from 1cm-7cms.

Cut section: Pale and glistening. Some tiny visible cysts range from 1-2mm in diameter.

MICROSCOPY: Sclerosed, collagenous tissue surrounding ill defined acinar and ductal lobules with cystically dilated duct structures. Staining with myoepithelial markers shows the presence of myoepithelial cells that surrounds the lobules and ducts.

DD: Adenocarcinoma NOS: Acinic cell carcinoma, Cystadenoma.

Presence of peripheral myoepithelial layer, lack of evidence of infiltration and lobular nature should prevent confusion from its mimickers.

ETIOLOGY OF SALIVARY GLAND NEOPLASMS [10,11,15.]

In general little known about the cause of salivary gland neoplasms. Many factors have been implicated in the causation of salivary gland tumor.

1. **VIRUSES:** The Epstein-Barr viruses and possibly auto-immunity leads to salivary gland carcinoma referred to as malignant lymphoepithelial lesions. Studies on human pleomorphic adenoma shows the presence of SV40 (Simian virus 40) DNA sequences and SV40 large T antigen.

2. **RADIATION:** An association between high dose radiation and salivary gland cancer is seen in studies of atomic bomb survivors. Iodine[131] used to treat thyroid disease also concentrated in salivary glands and increases the risk of salivary tumors.[26.]The risk of malignant salivary tumors is increased in patients of childhood cancer in head and neck region, treated by a combination of chemotherapy and radiotherapy.[27] Recent study of a population shows a higher frequency of benign parotid tumors, in a population of higher than average mobile telephone users[28].

3. **OCCUPATION:** Certain occupation has been reported to place people at an increased risk. These include asbestos mining, manufacturing of rubber products and industries such as shoe manufacturing, plumbing(exposure to metals) and working in automobile industries.

In Quebec there was an increased risk of salivary cancer among people living close to asbestos mines.[29]

4. **LIFESTYLE AND NUTRITION: John A et al** reported close association between cigarette smoking and Warthin's tumor.[10] The incidence is eight times that of non smokers. There is high risk of bilateral tumors in heavy cigarette smokers[8]. Exposure to kerosene and silica dust as a cooking fuel appeared to increase the risk in Chinese population.[30.]
5. **HORMONES**: High levels of progesterone receptors have been reported in recurrent PA.[31.] Progesterone receptors are found in acinic cell carcinoma and MEC, but not in salivary duct Carcinoma. 90% of salivary duct carcinoma shows Androgen receptors[10].

HISTIOGENESIS AND MORPHOLOGY OF SALIVARY GLAND NEOPLASM

Salivary gland have the greatest diversity of morphologic features among their cells and tissue. Although salivary, sweat, apocrine, and mammary glands all have similar phylogeny and cellular phenotypes, many lesions are unique to salivary glands.

HISTIOGENIC ASPECTS:

It is stated that specific reserve cells or basal cells of the intercalated and excretory ducts are both responsible for neoplastic transformation.

The **intercalated duct cells** is suggested to be cell of origin for Canalicular adenoma, Cellular adenoma, Oncocytoma, Warthin tumor, Papillary adenoma, adenocarcinoma, acinic cell carcinoma.

Excretory duct reserve cell is suggested to give rise to intraductal papilloma, PA, epidermoid carcinoma of salivary gland origin and Mucoepidermoid carcinoma..

The theories of tumor origin are based on relationship to salivary gland embryogenesis and potentialities of ductal epithelium during reactive process.[32] Regardless of cell of origin for salivary gland tumors it is essential to appreciate the types of cell differentiation, tumor cell organization,material synthesized by the cells and their placement within the tumor.

PLEOMORPHIC ADENOMA[10,11,13]

PA (mixed tumor, benign mixed tumor): Benign salivary gland neoplasm composed of ductal epithelial and myoepithelial cell proliferation within a mesenchymal stroma.According to Frazell and Foote,[35] the term mixed tumor dates from Minssen's in 1874 which is cited by Ahlbom.[36.]

INCIDENCE: 60% occur in parotid, 40-70% occur in submandibular and minor gland. The most common minor salivary gland site are palate, lip, and buccal mucosa. Other sites are cervical lymph node ,breast, lung ,mandible , pituitary fossa, lacrimal gland, sac, ear. Histologically it is similar to tumor of skin-**chondroid syringoma.**

Gender/ Race: Female > Male. (1.9:1)

AGE: 4^{th} – 5^{th} decade

CLINICAL FEATURES: Asymptomatic, slow growing mass. May become very large if neglected

GROSS: well circumscribed, variable encapsulated mass usually arise from superficial lobe especially lower pole. 10% arise in deep lobe. Cut surface is white tan, shiny or translucent. Mucoid tumors shows absence of capsule in some cases.

HUMARA (human androgen receptor gene) molecular assay shows that the stromal and epithelial cells of pleomorphic adenoma have a common origin.[11]

MICROSCOPY: Shows admixture of mesenchymal and epithelial structures. Epithelial element is composed of luminal ductal structures surrounded by myoepithelial cells. Stromal or mesenchymal elements are composed of myxoid, chondroid and hyaline areas.Rarely bone may form directly by stromal osseous metaplasia . PA in minor glands shows fat cells in the stroma , when the lipomatous component is 90% or more it is called-**lipomatous pleomorphic adenomas**.

CELLULARITY OF PA: when densely packed solid sheets of epithelial or myoepithelial cells occurs it is called- **cellular pleomorphic adenomas**.

IMMUNOHISTOCHEMISTRY[13]: Luminal cells of tubuloglandular element of PA are positive for CK 3,6,7,10,11,13,16. Pan-CK, CK 13,14,16 are irregularly positive for modified myoepithelial marker. There are also positive for vimentin, S-100 and GFAP. Currently the more reliable marker for myoepithelial cells are p63/Calponin which shows intense positivity. C-kit(CD-117) stains the luminal cells of PA.

MOLECULAR GENETICS: Cytogenetics shows recurrent chromosomal aberrations in approximately 70% of PA.

The targeted genes on ch8q and 12q are PLAG1 and HMGA2(formerly known as HMGIC) which encodes for transcription factors. Translocations resulting in overexpression of their genes has been postulated to play an important role in pathogenesisof pleomorphic adenoma. This can be detected by RT-PCR or fluorescence in situ hybridisation.

DD:

1. Monomorphic adenoma,
2. Adenoid cystic carcinoma
3. Epithelial myoepithelial carcinoma
4. Polymorphic low-grade Adenocarcinoma
5. Mucoepidermoid carcinoma
6. Various mesenchymal tumor such as nerve sheath tumor, smooth muscle tumor.

MYOEPITHELIOMA:[10,11,13] It accounts for 1-2% of all salivary gland tumors. Parotid is the most favorite site. It presents clinically as painless mass.

MICROSCOPY: Shows sheets and cords of cells which may be may be spindle, plasmacytoid, epitheloid, or clear cell in a collagenous or myxoid stroma.The spindle cell type shows fascicular pattern of growth.

Three morphologic type are

1. Spindle,
2. Hyaline(plasmacytoid) and
3. Mixed cell types.

The mixed cell type contain spindle and plasmacytoid cells in roughly equal part. Positive staining for calponin, actin and p63 indicate that the cells shows myoepithelial differentiation. Epithelial cells are large polygonal cells with eosinophilic cytoplasm and centrally located bland nuclei. They commonly show a lenticular, trabecular or solid growth pattern.

IHC:[13,19] clear cells are rich in glycogen. Pan-CK as well as myoepithelial markers(calponin, S-100, GFAP, Actin, CK-14, p63) are generally positive and usually negative for CK-7,EMA, and CEA.

BASAL CELL ADENOMA(BCA)[10,11,13.] It was first described by **Kleinsassar and Klein in 1967.[37] Various patterns described are**

1.Tubular 2.Trabecular, 3 Membranous.4.solid.

It accounts for 1%-7.5% of all salivary gland tumors, mainly in minor salivary glands. Peak incidence: 7th decade of life.

Parotid is the dominant site for occurrence of BCA. 75% occur in parotid followed by salivary gland in 5%. Upper lip being most common site in minor glands. It presents mostly as a painless mass of long standing duration.

GROSS: Round to oval, firm, smooth and encapsulated. Most tumors are <3cm in diameter.

C/s: grey to pink appearance often with sometimes cystic spaces can be seen. Membranous types are more often multinodular or multifocal.

MICROSCOPY: BCA shows small cells with scant cytoplasm with peripheral palisading giving a basaloid appearance . Polygonal basaloid cells with slightly more eosinophilic cytoplasm in tumor center which are arranged in trabeculated , tubular, membranous pattern and solid pattern.

The presence of ductal lumina among the basaloid cells are considered as tubulo-trabaculae type .The membraneous and trabecular type have a "jig-saw puzzule appearance".

Squamous differentiation in the form of whorls or eddies, cystic change and rare cribriform pattern are common. Tubular type are rarely oncocytic .

In adenoid cystic pattern multiple pseudocystic formation may sometime be observed in basal cell adenomas in which dilated lumina usually contain Alcian blue positive material.

IHC:1) CK positive in luminal and abluminal cells in all tumors 2)EMA: expressed in all tumors in apical portion of luminal cells. SMA, calponin, p63, S-100 are strongly expressed in spindle shaped stromal cells.

DD: 1)PA, 2)AdCC, 3)Canalicular Adenoma

Prognosis: 25% of membranous type have recurrence. Rare malignant transformation of BCA can occur.

WARTHIN'S TUMOR: was first reported by **Hildebrad** in 1895[38].It is composed of oncocytic epithelium and bilayered columnar cells that form multiple cysts. Sometimes accompanied by numerous papillary proliferation of follicle containing lymphoid follicle.

Synonym: 1)Adenolymphoma 2)papillary cystadenoma lymphomatosum.

EPIDEMIOLOGY: 2nd commonest benign parotid gland accounting 4-25% of all salivary gland. 5-7.5% of Warthin's are bilateral.[33,34.] Due to increased risk of smoking in females, the male predominance has now reduced to a 1.1:1 and 1.6:1.

AGE: Average 62yrs.

ETIOLOGY: Arise from trapped intraparotid or periparotid lymphoid tissue, intranodal occurrence suggest the possibility of multicentric growth. Smokers are at risk of eight times developing Warthin's compared to non smokers.

Gallo O et al 1997 stated that warthins is associated higher incidence of autoimmune disease.

CLINICAL PRESENTATION: Size: 2-4cm, Duration : 21month, pain 6% of cases, painless fluctuant swelling in the lower part of parotid. Technetium 99^{m} Pertechate concentrates in Warthin's tumor.

GROSSLY : Well circumscribed ovoid to spherical mass.

CUT SURFACE: Number of cysts with filled with brown viscous fluid or caseous debri. Between cystic areas minute tan to white nodular foci are seen. Coagulated content of cyst leave a rubbery consistency.

HISTOPATHOLOGY:

Tumor is sharply demarcated from surrounding parenchyma.It shows a cystic or epithelial bilayered lining with oncocytic features. The epithelium overlies a dense lymphoid component that forms germinal centers. PTAH(Phosphotungistic acid hematoxylin) demonstrate the granularity due to abundant mitochondria. Mucous, squamous or sebaceous cells are sometime seen. Mantle zone and germinal centers are found in more than half of cases.

IHC: CK- positive in luminal and basal epithelium. p63 stains the basal cells. No dominant B or Tcell clonal population are present.

Prognosis: 2% or less reccurence. Some recurrences are multifocal.

DD: salivary gland lesion with papillary oncocytic epithelial component. 2) Epithelial lesion with abundant lymphoid tissue. 4) Lymphoepithelial cyst.

ONCOCYTOMA (syn:Oxyphil Adenoma, Mitochondrioma, Oncocytic adenoma).

Hamperl et al defined it as benign tumor composed of large granular, eosinophilic polygonal cells with numerous atypical cytoplasmic mitochondria. Neoplastic transformation of oncocytic duct epithelium and acinar cells is considered to be the cell of origin

EPIDEMIOLOGY: occurs in elderly patient. No sex predilection but clear cell type is common in females.. Oncocytomas constitutes about 1% of salivary gland epithelial neoplasm. More common in parotid gland(80%). Exposure to radiation is seen in 20% of patients as reported by Brandman[38] et al.

CLINICAL FEATURES: Present as small firm slow growing nodular painless swelling sometimes bilateral in parotid.[39]

Gross : Firm, circumscribed, round to ovoid with slight lobulation.

C/S : Pink and uniform

MICROSCOPY: Composed of light oncocyte with small number of pyknocytic cells arranged in nest, trabeculae, sheets and duct like structures. occasional clear cells due to margination of mitochondria and intracytoplasmic glycogen can occur[12]. Rarely FNAC site produces squamous metaplasia. The clear cells stain with PAS and tumor cells stain typically with PTAH.

IHC: Immunoreactive for CK and antimitochondrial antibody.

Prognosis : Recurrence is rare. when it recurs it present as bilateral and multiple nodule. The incidence of bilaterality is 7%

DD :

1) Acinic cell carcinoma,

2) clear cell carcinoma,

3) MEC with prominent clear cell with alteration,.

CANALICULAR ADENOMA:[12,13,15] Benign, epithelial neoplasm, composed of interconnecting cords of columnar epithelium in an extremely loose stroma

EPIDEMIOLOGY : Occurs more common in female in 7th decade of life and constitutes 2% of all salivary gland neoplasm. 10% in palate, 16% in buccal mucosa 80% occur in upper lip.

GROSS: well circumscribed,tan to pink with solid or cystic cut surface.

HISTOPATHALOGY:shows tubules or strands of columnar epithelial cells situated opposite to each other .The epithelial cells are cuboidal to columnar with eosinophilic cytoplasms. Stroma is loose that appears to be "floating in the air"[13].

IHC : Shows immunoreactive for S-100 protein,CK7, CK8, CK13, CK14, and CD117.

SEBACEOUS ADENOMA[10,12,13,15.]

These tumors are rare occurs between 60-80 years of life,the commonest being parotid .

CLINICAL PRESENTATION : Size <3cm in diameter. Asymptomatic, firm to hard, slow growing mass.

GROSS: Cystic, circumscribed and white to yellowish in color.

HISTOPATHOLOGY: composed of cuboidal, basaloid, squamous and columnar cells arranged in island and small to medium sized cystic ducts that have numerous nests of sebaceous cells. Mucous,oncocytic metaplasia occur rarely

DD: 1) sebaceous adenocarcinoma, 2) Lymph adenocarcinoma .

SEBACEOUS LYMPHADENOMA

Age: 6^{th}-8^{th} decade, 90% occur in parotid, very rarely benign tumor.

Gross: Shows solid,yellow to grey multicystic or unicystic encapsulated mass .Sebum is found in many cysts.

MICROSCOPY: sebaceous glands of various sizes are surrounded by abundant lymphoid stroma. Foreign body reaction may present occasionally.

DUCTAL PAPILLOMAS: Rare benign salivary gland tumors arise from excretory ducts or junction between ductal and mucosal epithelium characterized by papillary growth. They are divided into 1) sialadenoma papilliferum, 2) intra ductal papilloma 3)) inverted ductal papilloma.

SIALADENOMA PAPILLIFERUM:

Age: 59yrs M > F. Podoshin et al described this lesion in a 35yr old women in parotid gland.

MICROSCOPY: Shows papillary process with convoluted clefts and spaces in between. Fibrovascular core are covered by parakeratotic, acanthotic stratified squamous epithelium. Double layer columnar to cuboidal epithelium cells overlies basal cuboidal cells. Chronic inflammatory infiltrate are frequent.

IHC: Luminal cells are positive for pancytokeratin. CK7, CK18, CK19, S-100 protien and EMA . S-100 protien, CK7, CK14 are reactive for basal ductal cells..

INVERTED DUCTAL PAPILLOMA: White and collegues in 1982 described this and it occurs in age group 30-60 yrs. Mean age is 60yrs. Both sexes are equally affected. The common sites are the minor salivary glands.

GROSS: Arise from the excretory duct of salivary gland and resembles sialadenoma papilliferum. They won't produce papillary fronds and may produce budding of mucosa.

MICROSCOPY: shows well circumscribed tumor mass within lamina propria and has an epidermoid appearance.

INTRA DUCTAL PAPILLOMA: occurs in minor salivary glands. Age : 29-77yrs. Mean age : 54yrs, equal sex ratio.

Gross: show cyst wall with partially or completely filled with friable tissue

MICROSCOPY : Papillary proliferation of bland columnar to cuboidal epithelial cells. It has a fibrovascular core that protrude into dilated cystic space[40].

CYSTADENOMA : Benign neoplasm characterized by adenomatous proliferation with formation of multiple cystic spaces lined by papillary projections.

Mean age: 8th decade, equal prevalence among male and female. 65% cases occur in major salivary, remaining 35% cases occur in minor salivary glands

GROSS : Tumor is well circumscribed with cystic spaces.

Microscopy : Encapsulated, well defined tumor with multicystic spaces. Epithelial lining may be flat, columnar or cuboidal. Oncocytic and mucus changes of lining epithelium may exist. Cell lining the cyst may produce papillary projection with central cores of connective tissue[41].

MALIGNANT TUMORS:

MUCOEPIDERMOID CARCINOMA[MEC]:[10,11,13.] Malignant epithelial glandular neoplasm characterized by intermediate, epidermoid cells and mucous cells. This account for 2-16% of all salivary tumor**. Stewart et al** in 1945 first described this as epidermoid tumor. MEC is unique that it exhibit various spectrum from indolent to aggressive course that are prone to recurrence, metastasis and local invasion[43].

AGE DISTRIBUTION: 3rd – 6th decade with mean patient age of about 45yrs. In children it account for the most common malignant gland tumor. Most of them are low grade with slight female predominance of 3:2 female to male ratio.[42]

Parotid being the most frequent site. Palate is the most frequently involved minor salivary gland site. Radiation exposure is the most common etiologic factor associated with development of MEC. It also reported in patient after radiation treatment for leukemia, lymphoma, sarcoma or thyroid Carcinoma.

Gross: Circumscribed but incompletely capsulated or unencapsulated firm mass. High grade tumor have infiltrative borders often with fixation to adjacent tissues.

Cut section reveals cystic structures accompanied by grayish white to tan solid areas. Size ranges from <1cm to over 12cms. In minor salivary gland the size measures about 5cms.

MICROSCOPY: MEC is composed of varying proportion of mucous,intermediate and epidermoid(squamous) cells. Intermediate cells are predominate with size between basal cells and epidermoid cells. Epidermoid cells have vesicular nuclei,open chromatin and abundant eosinophilic cytoplasm Occasionally individual cell keratinization or keratin formation are seen.

Mucocytes will take up the stains of mucicarmine and Alcian blue.They occur singly or in clusters cells, with a well defined cell membrane and foamy Cytoplasm. The intermediate, epidermoid and clear cells usually predominant in high grade with high reccurence rate and cervical lymph node metastasis. The low grade have predominantly cystic with few tumor cells.

Griddin et al reported sclerosing variant characterized by an intense central sclerosis[44]. some clear cell MEC's are associated with extensive intratumoral calcification[48] Some cases of MEC shows melanin pigmentation[49]

Special stain: Mucous cells are large and have pale to slightly basophilic fine cytoplasm with positive reaction to PAS, Alcian blue and mucicarmine stains.

Various grading system are suggested by different authors. WHO in its 2nd edition divided this tumor into two grades – well differentiated or low grade tumor and poorly differentiated or high grade. The 3rd edition WHO's grading system divides MEC into 3 grades[15.]

Histopathologic features	Point value
Neural invasion	+2
Cystic component <20%	+2
4 or more mitosis/10 HPF	+3
Necrosis	+3
Anaplasia	+4

Grade of tumor	score
Low	0-4
Intermediate	5-6
High	7 or more

GENETICS :

t(11,19) ,(q21,p12-13) translocation is frequently seen in MEC..

Molecular analysis demonstrates fusion transcript between MECT1 (Mucoepidermoid carcinoma translocation1) with MAML2 (master mind gene familyL2).This fusion transcript act as a co-activator for notch receptor transcriptional activation and signalling.[10,22] This translocation is frequently associated with low grade histology.

IHC:[13,20,52] Most tumor cells are positive for Pan- cytokeratin. Variable staining for EMA, CEA, and S-100. p63 staining is demonstrated in squamous and intermediate cells .

MUC1 and MUC4 are expressed by normal ductal cells.

MUC5B taken by acinar cells which have cytoplasmic mucin.

In high grade MEC, **MUC5AC expression is high which helps to differentiate it from Squamous cell Carcinoma**

High MUC1expression is correlated with high recurrence ,metastasis and short survival rate

High MUC4 is associated with **low grade tumor**, **low recurrence rate and long** survival rate . **Hence MUC4 is used in tumor differentiation grade**.[52]

In contrast to Squamous cell Carcinoma MEC often **express CK7 and negative for CK20.**

PROGNOSTIC FACTORS: Behavior of MEC is strogly correlated with clinical stage and histologic stage. Cure rate is high in low and intermediate MEC. Mayo clinic study shows that grade and stage are less important if radical surgery is performed.[45]

Tumor is considered to have low radio sensitivity. High proliferative index(mitotic count >2/10 HPF) or Ki index > 10%, expression of MUC1, vascular invasion, involved margin are associated with poor prognosis.

DD : 1)Warthin's tumor with squamous/mucinous metaplasia 2)Pleomorphic adenoma with squamous differentiation 3)poorly differentiated adenocarcinoma 4)Squamous cell Carcinoma.

ADENOID CYSTIC CARCINOMA[AdCC][10,11,13,20]

Definition : It is an aggresive type composed of basaloid cells with myoepithelial/basal cell differentiation admixed by ductal structures. It is characterized by tubular, cribriform or solid pattern of growth and a myxohyaline stroma. Adenoid cystic Carcinoma is encountered from 1-9th decade of life.

F:M Ratio is 3:2, higher incidence in 40-60yr of life. 42% arise from major salivary glands. 68% from minor glands. Most common site were parotid(21%), palate(17%), submandibular gland(13%) and it account for 10% of salivary epithelial neoplasm.

Billroth first described Adenoid cystic Carcinoma in1859. The term cylindroma was used until Frozell and Foote in 1953 expressed their preference for Adenoid cystic carcinoma.

In 1971 Hubner et al said that Adenoid cystic Carcinoma originate from myoepithelial cells claiming that acellular hyaline material is the product of myoepithelial cells.

In 1986, Chaudhry et al after their ultra structural of 12 cases suggested the origin from acinar- intercalated junction in the intercalated ducts proper[46].

Gross : Tumor is fleshy, firm, tan and invasive. Perineural invasion is very common.

Three characteristic growth pattern are defined,

1) **Cribriform pattern** is the most frequent pattern observed, composed of two main types 1) Ductal 2) Myoepithelial cells with microcystic spaces. They have typical hyperchromatic angular nuclei with clear cytoplasm. They have propensity to involve the peripheral nerves[47].They are PAS +ve for hyaline or basophilic mucoid pattern.
2) **Tubular pattern** shows luminal layer of epithelial and outer layer of myoepithelial cells , characterized by well formed tubules and ducts with central lumina
3) **Solid or basaloid** types shows sheets of uniform basaloid cells but lacks tubular or microcystic formation.

Perzin et al in 1978 concluded that in soid type prognosis is poor with high incidence of metastasis and rapid clinical course. The cribriform pattern has intermediate prognosis and tubular pattern having best prognosis.

Paulino AF et al studied 29 cases and stated that AdCC characterized by infitrative capacity and propensity for neural invasion. Facial nerve palsy and large area of tumor necrosis are bad diagnostic signs. Dedifferentiation can also occur in Adenoid cystic Carcinoma,[50] such cases caries very poor prognosis. Brain derived neutrophilic factor is a growth factor involved in neurogenesis and is uniformly expressed[51.]

HISTOCHEMISTRY: The luminal spaces with eosinophilic hyaline material take up PAS/Alcian blue.

IHC : Ductal lining cells are positive for C-Kit. Epithelial cells are variably positive for it markers such as CK, CEA, EMA. Myoepithelial cells will stains for p63, Calponin, S-100. GFAP positivity suggest neural invasion and late distant metastasis. Ki-67, C-Kit help to differentiate between PLGA and AdCC.

Prognostic factors: large size >2-4cm, minor glands, High Ki67 index shows poor prognostic factor.

ACINIC CELL CARCINOMA[ACC][10,11,13.]

Definition : It is a neoplasm demonstrating at least focal differentiation towards serous acinar cells. **Buxton et** al in 1953 were the first to describe the term serous cell adenocarcinoma. Tumors constitutes 4-6% of all salivary neoplasms.

Commonest site : Parotid and minor percentage found in submandibular and minor salivary gland. Rarely parapharyngeal spaces,[53] pancreas and lung.

In 2002 **Hwei-Yee,et** al a primary ACC of lung with regional lymph node metastasis and perineural invasion.[54.]

Age : 3rd –7th decade. Mean age is 44yrs. Second most common malignancy in children. Female predominance has been observed.

Gross : Well circumscribed encapsulated mass with a diameter of 2-4cm. Grey white to reddish gray on cut section. Consistency varies from firm to soft. Most of the tumors are solid and cystic tumors.

Microscopy: These tumors shows acinar cell differentiation with zymogen granules in cytoplasm. Pattern of growth varies from solid microcystic, papillary cystic, follicular, tubule ductal or dedifferentiated and non specific glandular. A delicate fibrous pseudocapsule and psammoma body like calcification and a may seen in some tumor.

CLASSIC ACC OR THE SOLID VARIANT: Shows well differentiated serous acinar cells with basophilic to gray granular in the cytoplasm. PAS +ve and mucicarmine –ve zymogen granules are seen in the cytoplasm. In high grade malignancy vascular and neural invasion are seen.

PAPILLARY- CYSTIC VARIANT: Rare variant characterized by large cystic spaces which are lined by stratified cuboidal or simple epithelium with some papillary projection. The papillae are covered by hobnail cells, intercalated duct like cells, vacuolated cells and non descript variant cells which posses eosinophilic to amphophilic cytoplasm, central nuclei and indistinct cell borders.

FOLLICULAR VARIANT: Closely packed round cystic spaces filled with homogenous eosinophilic colloid- like material highly reminiscent of thyroid follicles. The colloid materials are PAS +ve.

DEDIFFERENTIATED ACINIC CELL CARCINOMA: Associated with rapid tumor growth, facial pain, bulky tumor and extremely poor prognosis. Usually present with areas of poorly differentiated adenocarcinoma NOS type. Extensive oncocytic change has rarely reported.[55]

IHC: Acinar cell and ductal cells are positive for CK, CEA, amylase. DOG(discovered in GIST) – recently shown to be in acinar cell markers.[56] Prognostic factors clinical stage and resection margin status are important prognostic indicators. Simpson et al[57] suggest that dense lymphoid stroma with germinal centre and microcystic growth pattern have particularly favourable prognosis.

POLYMORPHOUS LOW GRADE ADENOCARCINOMA[10,11,13,15.]

In 1984 Batsakis and Evans first described this carcinoma. WHO classification of salivary gland tumor adopted the term polymorphous low grade adeno carcinoma.

CLINICAL FEATURES : The palate is the most common site(60-70%), followed by buccal mucosa (16%) upper lip (12%) retromolar area, base of tongue rarely lacrimal gland. Peak age is in 5^{th}-6^{th} decade. F>M, present as asymptomatic mass with or without ulceration.

GROSS : Circumscribed, non encapsulated, tan to gray masses with glistening cut surface with infitrative margins. Size 0.4-6cm with average of 2.2cm. Perineural invasion is common.

MICROSCOPY: Tumor has polymorphous growth architecture showing predominantly solid, tubular, fascicular and cribriform foci of squamous are mucinous metaplasia or with areas of papillary change are found.[58] The tumor cells have round pale nuclei with fine evenly spaced chromatin and indistinct nucleoli. The tumor cells always maintain bland cytological features with cuboidal, spindled or polygonal shapes.

Mucinous or clear cells are occasionally seen. Myoepithelial cells are absent or at most present very focally at the light microscope level. Stroma is slight grey blue and is said to be characteristic. Psammoma bodies and collagenous or tyrosine type crystalloids are found in some cases.

Dedifferentiation to high grade salivary duct carcinoma with predominantly solid and cystic growth, high nuclear grade, obvious tumor necrosis present.

IHC:[19,20] Positive for low and high molecular CK, EMA. Strongly and diffusely positive for S-100 protein. Some but not all PLGA are also positive for myoepithelial markers. Ki67 index for PLGA is 6.4% when compared to 54% of high grade Adenoid cystic carcinoma help to different both.

AdCC were strongly positive for C-Kit but only small percentage shows positive for PLGA

DD : 1) PA 2) Adenoid cystic carcinoma.

EPITHELIAL-MYOEPITHELIAL CARCINOMA (EMC)[10,11,15.]

Donath and colleagues in 1972, first described this carcinoma. Rare tumor 0.4% of salivary gland neoplasm. Peak incidence : 6^{th}-7^{th} decade. 60% percent occurs in parotid.

CLINICAL FEATURES : Asymptomatic mass. Patient present with pain and facial weakness. It is a low grade tumor with recurrence of 30-40%. Regional metastasis occurs in 10-20% of cases. Distant metastasis to kidney, lung and brain is uncommon.
Gross : Well defined swelling, nodular or multi nodularity $1/3^{rd}$ cases are encapsulated. Cut surface shows grey yellow to white with cystic areas. Tumor measures 2-8cms in diameter with mean 3cms.

MICROSCOPY : EMC is characterized by double layered, duct like structures lumid cells are arranged as single cuboidal or columnar luminal cells that have a central round or oval bland looking nucleus with moderate amount of pink cytoplasm. Rarely extensive squamous, sebaceous or apocrine differentiation can be present. Perineural and vascular invasion are common . Moderate to severe degree of nuclear atypia is seen in dedifferentiated tumor.

Special stain in EMC : Clear cell components contain abundant glycogen which shows PAS positive material .

IHC: Ductal cells are positive for Pan-CK, variably stain for S-100 protein but do not take up the myoepithelial markers. Abluminal cells are positive for p63, S-100 protein, calponin and actin. Proliferative index is low <1% for ductal cells and <3% for abluminal cells.

DD : Distinguished from other clear cell tumors with double layered as duct like structure. 1) Pleomorphic adenoma. 2) Tubular variant of AdCC. 3) Mucoepidermoid carcinoma 4) Acinic cell carcinoma.

PROGNOSIS : Marginal status is the most significant pathologic prognostic factors. Poor prognosis is seen in tumors located in minor salivary and seromucinous glands because of high frequency of recurrences.

SALIVARY DUCT CARCINOMA

In 1968 Kleinsasser et al first described salivary duct carcinoma.

Definition : Aggressive malignant tumor morphologically resembles high grade ductal carcinoma.

Clinical features : Age : 6^{th}- 7^{th} decade. Sex M : F = 3 to 6 : 1. Parotid account for 80% of cases. Patient present with rapidly growing parotid mass associated with facial nerve palsy (42%), pain (23%) and 35% shows cervical lymphadenopathy. It is the most aggressive tumor with a mortality rate of 77%. They account for 2% of salivary neoplasms.

Gross : Unencapsulated,solid, highly infiltrative grey to white with rare areas of cystic change and foci of necrosis. Size : 3cm.

MICROSCOPY : Infiltration into soft tissue and adjacent gland is evident. Histologically mimicks high grade ductal Ca of breast with mixture of two components

- **INTRA DUCTAL :** consist of large ductal structures with various pattern 1) cribriform, 2) papillary and 3) solid growth pattern.

cribriform carcinoma: may form tumor cells arches in relatively large intercellular spaces called 'roman bridge architecture'. Comedo necrosis is common in

the central portion of ductal cells. Psammoma bodies and focal squamous differentiation, some with keratinization can be seen in tumor nests cells.

- **INVASIVE COMPONENT :** shows irregular cords and glands that frequently shows desmoplastic reaction. SDC with pure intra ductal or minimally invasive form associated with better prognosis. Neoplastic cells have apocrine appearance with abundant eosinophilic cytoplasm, large pleomorphic vesicular and prominent nucleoli. Stroma densely fibrous or desmoplastic, perineural invasion, intravascular tumor emboli are common.

Variants of SDC :

1) **Sarcomatoid variant(Dedifferentiated salivary duct Ca):**[82,83]Composes of anaplastic spindle cells, multinucleated giant cells, rhabdoid cells, osteosarcomatous cells. This is consider as a dedifferentiation of salivary duct Carcinoma.
2) **Mucin rich variant:**[84] Clusters of carcinoma cells float in a area of mucin or colloid.
3) **Invasive micro papillary variant:**[85.] Shows morule like tumor cells with absence of fibrovascular cores surrounded by clear space morphologically similar to micropapillary variant of breast .
4) **Oncocytic variant** : Abundant eosinophilic granular cytoplasm and strongly immunoreactive with anti mitochondrial antibody.

Prognosis: Brandwein et al stated that Size <3cm are have good prognosis.[88] Histologically micro papillary pattern are more aggressive. Lymph node metastasis and positive surgical margins associated with less survival rate.

IHC : Tumor cells shows diffuse strong staining for CK, EMA and CEA.

Fan CYet al in 2000[86] stated that almost all cases express Androgen receptor, ER/PR are usually negative. HER-2/neu over expression is associated with worst prognosis. Some cases shows prostatic acid phosphatase, prostatic specific antigen or CK20. Ki-67 index is high (mean 21.3%).

Laforga et al in 2004 reported a single case with neuroendocrine differentiation[87].

Genetic : Loss of heterozygosity is detected in 9p21, 6q, 16q, 17p gene. Mutation and over expression of TP53 gene and protein are frequent. HER2 gene amplication occur in 12-36% and over expression in 26-100% of cases. Gene expression profiling stated by Leivo et al in 2005 stated overexpression of apoptosis related gene CASP10[Caspases-10] and MMP11[matrix metalloprotein-11] in SDC.[89] Tumor progression is associated with inactivation of CDKN 2A/p16 gene.[Cyclin Dependent Kinase inhibitor 2A].

DD : 1) Metastatic breast / prostatic Ca 2) High grade MEC 3) Oncocytic Carcinoma 4) Cystadenocarcinoma 5) Intraductal Carcinoma.

INTRA DUCTAL CARCINOMA.

Synonym: Low-grade salivary duct Ca/low grade cribriform cystadeno Carcinoma. In 1983 Chen first described intraductal Carcinoma, later it was defined as tumor of low malignant potential similar to mammary counterpart[90].

Clinical features : Frequently affects the parotid (mean age of 62yrs) with slight female predilection. Minor salivary gland (palate, tongue,and oral cavity) can be affected.

MICROSCOPY : Smooth-contoured ducts with epithelial cell proliferation forming solid–comedo, cribriform, micropapillary or Roman bridge pattern similar to salivary duct Carcinoma. Focal invasion into surrounding tissues is seen.

CLEAR CELL CARCINOMA : not otherwise specified[10,11,13.].

Malignant epithelial tumor occurring in 40-70yr of age. Frequently occur in minor salivary glands, clinically with mucosal ulceration and pain.

Gross : <3cm, poorly circumscribed, infiltrating into adjacent mucosa, soft tissue, bone and nerves with grey white on cut section.

Microscopy : Composed of nests and cords , sheets of monomorphous polygonal to round cells with clear cytoplasm and is characterized by absence of features characteristic of any other salivary gland tumor with clear cell change. Prognosis is excellent.

BASAL CELL ADENOCARCINOMA. [10,11,13,15.]

Low grade malignant neoplasm with cytological resemblance to basal cell adenoma. Diagnosis is confirmed with demonstration of infiltration of surrounding salivary lobules, nerves or blood vessels occur in average age of 60yrs with no sex predilection.

Arise de novo in the parotid gland less commonly of submandibular gland, oral cavity and upper respiratory tract[91]. In 23% of cases it arise from basal cell adenoma particularly the membranous types or monomorphic adenoma.

Gross : Most occur in superficial lobe of parotid gland. Cut surface is grey, tan white or brownish unencapsulated but may be well circumscribed but usually infiltrative.

Size : 0.7 – 7cm.

Basal cell adenocarcinoma has predominantly solid pattern,characterized by islands of tumor cells, areas of basaloid cells with peripheral palisading. 25 to 33% of these tumor may show intravascular and perineural invasion.[92]

IHC : Tumor cells are reactive for CK and locally reactive for S-100, EMA.

MALIGNANT SEBACEOUS TUMORS. [10,13,15.]

1) **Sebaceous carcinoma** : In 1966, **Goldstein and silver** first described as a rare primary carcinoma of salivary glands. Bimodal age distribution between 3rd and 7th decade. No sexual predominance. Usually present as painless mass with perineural invasion and majority of them occur in parotid gland.

Gross : Size : 0.6 – 8.5cm. Color varies from tan white, grey white to pale pink well circumscribed or partially encapsulated with pushing or infiltrative margins.

Histology : Tumor is composed of nests or sheets of sebaceous cells of varying maturity, different degree of polymorphism, nuclear atypia and invasiveness. Squamous and basaloid differentiation are common, with perineural invasion is seen in 20% of cases[93].

2) **Sebaceous lymphadenocarcinoma**: Rarest salivary gland sebaceous tumor with only 5 cases reported in the literature[93].

Age : Occur in 7th decade, arise in parotid or in periparotid lymph node.

Gross : Yellow tan to grey colored. Partially encapsulated with areas of invasive foci

Microscopy: composed of typical sebaceous lymphadenoma intermixed with or adjacent to regions of pleomorphic carcinoma cells exhibiting varying degree of invasion.

CYSTADENOCARCINOMA:[10]

Most of the patient are over 50yrs of age, 65% occur in major salivary gland as a slow growing, compressible asymptomatic mass.

Gross : numerous cystic spaces of varing sizes filled with mucin.

Microscopy : Well circumscribed, shows predominantly cystic growth with intraluminal, papillary pattern.[15]

ONCOCYTIC CARCINOMA[3,11.] **:** In 1953, Bauer described the first case. Constitutes < 1% of all salivary gland tumors. Males are affected in 2/3rd of cases.

Age : 25 – 91yrs with mean age of 62.5yrs. 80% occur as painless mass.

Gross : Unilocular or multilocular masses. Unencapsulated tan to grey, some cases are focally necrotic.

Microscopy: Cells shows variations in size, shape and nuclear pleomorphism. Minimal nuclear atypia. Tumor cells form nests or ducts, trabeculae, sheets that infiltrate salivary gland parenchyma and surrounding tissue. Perineural and vascular invasion can occur.

High Ki-67 help to differentiate it from oncocytoma.

ADENOCARCINOMA, NOT OTHER WISE SPECIFIED : It refers to primary carcinoma of salivary gland which exhibit glandular differentiation but lacks diagnostic

criteria of other tumor categories. Occurs in elderly. Peak age 6^{th} -8^{th} decade of life with male preponderance.

Site : Parotid gland, submandibular gland, palate and buccal mucosa. Facial nerve involvement is common. Tumor involving oral cavity carries favourable outcome than that of parotid or submandibular gland.

Gross : Firm, infiltrate and partially circumscribed with areas of haemorrhage and necrosis.

MICROSCOPY : shows ductal or glandular differentiation with invasion into adjacent salivary gland parenchyma. It lacks any of the histomorphologic features of other recognised salivary gland adenocarcinoma. Most adenocarcinoma NOS are considered to be high -grade histologically.

Immunohistochemistry: positive for pan–cytokeratin .

MYOEPITHELIAL CARCINOMA : Rare neoplasm occuring in elderly is composed predominantly of tumor cells with myoepithelial differentiation with tendency for metastasis and infiltrative growth. Clinically present as destructive tumor with pain or painless mass.

CARCINOMA EX PLEOMORPHIC ADENOMA[10,13,15][CaexPA] :

In 1957, Bearhrs et al first described carcinoma ex pleomorphic adenoma.

Definition : Carcinoma that arise from epithelial or myoepithelial component of pleomorphic adenoma.

M.sherif et al in 2013 stated that ca ex PA constitute 99% of all cases of malignant mixed neoplasms. It develops in 6% of all PA and constitutes 3.6 – 4% of all salivary gland neoplasms.. Occurs in 6^{th} -7^{th} decade of life. Median age of onset : 10 – 20yr older than patient with PA.

Hu et al suggests that p16 gene methylation may be responsible for evolution of PA to ca ex PA.[94]

Bassel Tarakji et al [95](2010) stated that inactivation of p53 play a important role in evolution of Caex PA. **Eiji Mitate et al** 2013 reported a case of an unusual malignant component of squamous cell carcinoma[96].

GROSS: poorly circumscribed and infiltrative masses to completely encapsulated appearing masses.

MICROSCOPY: The PA component was represented by scarring, the malignant component shows features of SDC,poorly differentiated carcinoma NOS type.

Genetic studies : Amplification and over expression of gene 12q13-15 including CDK4, HMG1C and MDM2 are important events in malignant transformation. HER2/neu over expression occur in 21–82% of cases. Altered p53 gene are found in 29-67% of cases and p53 over expression seen in 41 -75% of cases suggest that may play a role in transformation.[65,67]

Ki -67 proliferative index is increased compared with PA. S100, Her2/neu help to differentiate carcinoma ex pleomorphic adenoma from atypical PA[65,67.].

CARCINOSARCOMA[10,11,13] : Rare malignant tumor of salivary gland composed of combination sarcomatous and carcinomatous elements. It occurs in elderly as painful mass. Parotid gland is involved in 2/3rd of cases.

GROSS : Poorly circumscribed mass, occasionally tumors are partially or totally encapsulated.

Cut section : Greyish in color with yellowish area of necrosis and haemorrhage.

MICROSCOPY: The commonest sarcomatous elements are Osteosarcoma and chondrosarcoma. Ductal carcinoma is the most common carcinomatous element and they are treated with wide surgical excision.

METASTASING PLEOMORPHIC ADENOMA[13] : Rare complication of PA with metastasis occurring after 1.5 – 51yrs. 40 cases have been reported so far. 3/4th of cases occur in parotid gland. Metastatic tumors retains the benign histologic feature of pleomorphic adenoma. Common metastatic site are 1) bone (50%), 2) lung (30%) and 3) lymph node (30%). Scalp, abdominal wall and liver metastasis have been reported[97]

SQUAMOUS CELL CARCINOMA[10,13] : It arises from squamous metaplasia of salivary duct. 80% of cases occur in parotid of age group 7- 65yrs.

GROSS : unencapsulated, poorly demarcated, firm to hard in consistency.

CUT SURFACE: grey or white

MICROSCOPY : Similar to squamous cell carcinoma ranging from low grade, highly keratinized neoplasm to poorly differentiated sheets of tumor cells with minimal keratinization. Soft tissue invasion and regional metastasis are common

DD : 1) High grade MEC, 2) Metastatic carcinoma, 3)Primary salivary gland Squamous cell Carcinoma..

IHC: Squamous cell carcinoma were negative for MUC5AC,CK-7,CK-20 these markers helps to distinguish **high grade MEC** from Squamous cell Ca[60] which shows **CK-7 positive and CK-20 negative**..

SMALL CELL CARCINOMA : Very aggressive rare tumor account for <4% of major salivary gland tumor. These are common between 5th and 7th decade of life. 85% of small cell carcinoma arise from parotid and others from sub mandibular glands.More than half of patients develop local recurrences or metastasis[98].

Gross : Tumors are poorly demarcated. They are firm to hard in consistency

Cut section: Variegated appearance

Microscopy: shows proliferation of small pleomorphic cells with fine nuclear chromatin, inconspicuous nucleoli and with scant amount of cytoplasm resembling small cell carcinomaof lung[99,100.]

IHC : 73% of salivary gland small cell Ca express CK20[99].

LARGE CELL CARCINOMA: Rare tumor,occurring in parotid composed of pleomorphic cells with abundant amount of cytoplasm .

LYMPHOEPITHELIAL CARCINOMA: It is an undifferentiated Carcinoma similar to morphology of poorly differentiated ca of nasopharynx.. High prevalence among Chinese population. Present clinically as mass in the cheek since birth.[101]

SIALOBLASTOMA: Rare congenital epithelial tumor, **Vawter and Tefft** first described this case as "embryoma"in 1966 since then various names were used. It was locally invasive.

MICROSCOPY: Shows basaloid cells with very few nucleoli with very few cytoplasm.

IHC : CK positive in ductal component, Vimentin in both ductal structures and solid nests.[102]

SOFT TISSUE TUMORS :

Benign tumors: with exception of hemangioma, soft tissue tumors of salivary gland are very uncommon and mostly benign.they account for 1.4% of salivary gland tumor. Of this 30.5% are neural tumors, 30% hemangiomas, 18.5% are fibrous, 9% lipomas, 7% lymphangioma, 5% others. Hemangiomas and lymphangiomas occur during first three decade of life.

1) **Hemangioma** : Soft tissue tumor constitutes of 0.4% of all salivary gland neoplasm. Most common age group of presentation is 2nd decade, composed of proliferation of pericytes and endothelial cells .

2)**Lipoma** : uncommon benign soft tissue tumor involves parotid with incidence of 0.5-2% M : F ratio is 10 : 1. Subtypes like spindle cell lipoma, angiolipoma, and hibernoma have been reported.

3)**Lymphangioma** : rare tumor usually occur in parotid.

4)**Neurofibroma and Neurilemmoma** : It arise from one of the fine radicals of facial nerve.

5)**Sarcoma** : they are very rare. Fibrosarcoma and malignant schwannoma are the commonest. In 1997, Shat et al report one case of intra parotid Schwannoma.

Lymphomas : It arise in salivary gland, very rare as the only manifestation or part of systemic disease. Most common are NHL type (85%) most NHL's and B-cell lymphomas, follicular and extra nodal marginal zone B-cell lymphoma.

SECONDARY TUMORS:[10] Peak incidence 7th – 8th decade. It comprises 5% of all malignant tumor salivary gland tumors[103]. Majority occur in parotid followed by submandibular. Most frequent are small cell carcinoma followed by malignant melanoma. 84% of tumor that metastasize to parotid originates in head and neck region. Other sites are lung, kidney, and breast.

IMMUNOHISTOCHEMISTRY[19,20.]

ROLE OF IMMUNOHISTOCHEMISTRY IN SALIVARY GLAND TUMORS

Salivary gland tumors shows striking morphological diversity. Tumors contain various propotion of ductal/luminal cells and myoepithelial /basal cells. This provides the basis for immunohistochemical demonstration of various components. The main application of IHC in salivary gland tumor is to demonstrate the existence of myoepithelial/basal or luminal component when diagnosis is uncertain.

Many immunohistochemical investigation uses differentiation markers specific for myoepithelium. Initially S-100, vimentin ,and GFAP were used but found to be non specific. The most reliable marker currently used for neoplastic myoepithelial cells are p63,calponin and mapsin.

MYOEPITHELIAL MARKER- p63.

p63 gene, a member of p53 gene family play an essential role in epithelial development, stem cell identity cell and cellular differentiation.[73]

It is expressed in atleast six protein isoforms [70,71,76.].They are divided into two groups.

1. Those with transcription activation domain.(TAisoforms).
2. Those without transcription activation domain(Delta N isoforms).

TA isoforms are able to activate transcription of specific target and induce cell cycle arrest and apoptosis.Studies on human tumor suggest an oncogenic function for Delta N isoforms.

Foschini et al stated [71]that the isoforms of p63 was present in the tumoral tissue but absent in normal salivary gland.This observation suggest that p63 particularly its isoforms, is involved in the neoplastic transformation of salivary glands.

Weber et al[73] [2002] stated that in normal parotid tissue **the expression of p63 was restricted to few basal and myoepithelial cells.** Ductal,luminal,and acinus cells were completely negative Salivary gland tumors shows strong nuclear staining for p63,which suggest the role of p63 in oncogenesis of these tumors.

Bilal et al [75] stated that p63 is retained in modified myoepithelial and basal cells of human salivary gland tumors ,which suggest the role of p63 in the oncogenesis of tumor complex.

p63 is positive in PA, basal cell adenoma, warthin's tumor, PLGA ,epithelial-myoepithelial cell carcinoma and myoepithelial cell carcinoma.

Edward PC [72] stated that p63 is strongly expressed in basal cell adenoma of parotid origin . In Adcc positive reactivity is observed in the non luminal myoepithelial cells surrounding the luminal cells. In canicular adenoma p63 staining was found to be negative. p63 does not appear to be a ideal marker for distinguishing Adcc ,PLGA,and basal cell adenoma

p63 is also expressed in squamous epithelial cells, and basal cells, so care should be taken while evaluating them.

Other myoepithelial markers: calponin ,α-SMA, S-100,Vimentin are non specific markers because they are also observed in ductal cells. They are only used for initial screening of myoepithelial cells. GFAP has low sensitivity as a myoepithelial marker, but frequently detected in pleomorphic adenoma and myoepithelioma.

Langham G et al[65] stated WT1(Wilms tumor product) was recently reported as sensitive marker of neoplastic myoepithelial cells in pleomorphic adenoma.

Hence Panel of markers are used to investigate neoplastic myoepithelial cells. For cases with minimal number of section, screening with pan –CK,calponin, α-SMA, p63 and S-100 protein is the best in terms of specificity. Normal myoepithelial cells serve as internal control for myoepithelial markers.So, if possible IHC should be performed in a section with normal salivary gland tissue.

TUMORS WITH ONCOCYTIC AND SEBACEOUS DIFFERENTIATION.[65].:

Tumors with oncocytic differentiation such as warthin's, oncocytoma, oncocytic carcinoma, ,Mucoepidermoid carcinoma are positive for antimitochondrial antibodies. Tumors with sebaceous differentiation such as sebaceous adenoma and carcinoma are intensely positive for **EMA.**

McHugh J B et al [74](2007) stated that metastatic renal cell carcinoma in head and neck often mimicks benign and malignant oncocytic lesions. Expression of p63 in oncocytic lesions exclude metastatic RCC(Renal cell carcinoma) from the differential diagnosis of these salivary gland lesion.

DIFFERENTIAL DIAGNOSIS OF PROBLEMATIC SALIVARY GLAND TUMOR

AdCC,SDC exhibits cribriform structures , others in differential diagnosis are PA,EMC,PLGA, Sialoblastoma.

α-SMA/calponin positivity distinguish AdCC and BCA which are sometime challenging to diagnose by histological examination alone.

Ki -67 labelling index ≥10% in AdCC, whereas≤ 10% in BCA. Presence of strong S-100 positive stromal cells support the diagnosis of BCA. Sialoblastoma are differentiated by early onset of age.

TUMORS OF BENIGN AND MALIGNANT COUNTERPART:

Benign and malignant counterpart of salivary gland tumor shares similar histological features. Malignant counterpart is differentiated by perineural and vascular invasion, necrosis and mitosis. IHC assessment of Ki-67 labelling index help to differentiate myoepithelioma ≤10% from myoepithelial carcinoma ≥10%. IHC markers for BCA and basal cell adenocarcinoma displays a striking similarity so they are of little value in differential diagnosis.

- Higher rate of cell proliferation(Ki-67≥5%).
- Apoptosis index >0.4%.
- p53-strong positive.
- Loss of bcl-2 expression

Differentiates basal cell adenocarcinoma from basal cell adenoma. These tumors may positive for HER-2/neu and high Ki-67 index.

DIAGNOSIS OF SPECIFIC TUMOR TYPES:

PLEOMORPHIC ADENOMA(PA): Luminal cells are positive for cytokeratins. In myxoid and solid areas neoplastic cells are positive for vimentin, S-100, GFAP, calponin and smooth muscle myosin. p63 shows positive in modified myoepithelial cells. A recent study of 45 cases of pleomorphic adenoma revealed that all 45 were positive for PLAG1. PLAG1was also specific for CA ex PA against other tumors.

C-KIT(CD-117) IN ADENOID CYSTIC CARCINOMA:[19,20]

C-KIT : A transmembrane receptor tyronine kinase, is negative in normal salivary gland, but more than 90% of cases of Adcc are positive for CD-117 [luminal cells]. Recent literature stated that CD-117 is expressed in Adcc is not specific for Adcc[59]but the extent and intensity of staining exceeds other tumors.

Holst VA[78,79] et al stated[1999] that gene mutation of exon 11 or exon 17 is not a mechanism of c-kit activation in these neoplasms.

Carla R Penner D.D.S.et al[80][2002]study stated that c-kit is expressed in ADcc but not in PLGA. Expression of galectin -3 is significantly expressed in PLGA but decreased in Adcc.

Seethala RR,[105]et al [2004]study shows CD43, a marker of T- cell and histiocyte tend to be localized in abluminal cells in a membranous pattern. Thus CD-43 immunohistochemistry have some utility in supporting the diagnosis of Adcc in difficult cases.

MUCOEPIDERMOID CARCINOMA:(MEC)

p63 expression is absent or minimal in mucoepidermoid carcinoma. MEC express membrane bound mucin such as MUC1, MUC4, MUC5AC and MUC5B.

Handra –Luca A et al[104] [2005] stated that MUC1 is associated with high histological grade, metastasis and recurrence rate. MUC4 is associated with tumor differentiation with low histological grade, low recurrence. Positive staining for MUC5AC help to differentiate high grade MEC from squamous cell carcinoma. MEC that shows over expression of HER2/neu had lower survival rate[106].

SALIVARY DUCT CARCINOMA(SDC):

High grade malignant tumor similar to duct breast carcinoma. Androgen receptors are frequently positive in this tumor. About 80% of this tumor shows diffuse/strong membranous positivity for HER2/neu.[19]

Ryuichi Murase et al[107] stated [2011] that Androgen receptor[AR] expression in SDC patients are sensitive to hormone therapy.

A Etges et al[68] [2003] stated that HER2/neu is associated with poor prognosis and there was a correlation between HER2/neu oncoprotein expression and aggressive behavior in SDC.SDC are associated with high Ki-67 proliferative index.

CARCINOMA EX PLEOMORPHIC ADENOMA[65]

Immunohistochemically Ca ex PA are strongly positive for AR,p53, HER2/neu and high Ki-67 labelling index.[65] A recent study stated that S-100 protein play an important role in malignant transformation of ductal cells in pleomorphic adenoma.hence staining for S-100 would be a useful diagnostic marker in early phase of pleomorphic adenoma.[129] PLAG1was specific for CA ex PA against other neoplasms[65].

EVALUATION OF MALIGNANCY AND PROGNOSTIC FACTORS[65]:

Ki -67 most frequently reported prognostic factor in MEC, SDC, CA ex PA. MEC and AdCC shows low recurrence when prognostic index is <5% and associated with poor prognosis when Ki-67>10%.

p53 for AdCC and SDC and HER2/neu are considered as prognostic factors.

IHC: plays a limited, despite important role in diagnosis of salivary gland neoplasms. It is necessary to understand that IHC should be a method **to assist the final diagnosis but not change the histopathological based diagnosis**. .

OBSERVATION AND RESULTS

During the study period from September 2012 to August 2014, a total of 13,916 specimens were received for Histopathological examination, out of this 92 specimens were of salivary gland lesions representing 0.6% as shown in the [table1,2], [chart-1,2].

Table 1

Total number of specimens	13916	100
Other lesions	13824	99.4
Salivary gland specimens	92	0.66

Of the total 92 cases, 28 cases were diagnosed as non neoplastic , 64 cases as neoplastic lesions of which 41cases were benign and 23 cases were malignant.

Table 2:

INCIDENCE OF SALIVARY GLAND LESIONS

	SEP-2011 AUG-2012	**SEP-2012 AUG-2013**	**SEP-2013 AUG-2014**	**TOTAL**
NO OF SPECIMENS	5063	4505	4345	13,916
SALIVARY GLAND LESIONS	30	34	28	92
PERCENTAGE	0.59%	0.75%	0.64%	0.66%

Of the total 92 cases, 28 cases were diagnosed as non neoplastic and 64 cases as neoplastic lesions, of which 41cases were benign and 23cases were malignant as shown in table 2. Out of 41 benign cases, 30 cases were Pleomorphic adenoma, 6 cases were Basal cell adenoma, 4 cases were Warthin's tumor and one case of Myoepithelioma. The incidence of benign tumor was 64.06% which comprises of 44.56% of total salivary gland lesions.

Twenty three cases were diagnosed as malignant, of which 12 were mucoepidermoid, 5 were Adenoid cystic carcinoma. Salivary duct carcinoma, carcinoma ex Pleomorphic Adenoma constitutes about 2 cases each and basal cell adenocarcinoma and primary squamous cell carcinoma constitutes one case each.The incidence of malignant tumor was 35.94% which constitutes 35.94% of total salivary gland lesions.

Table 3:

INCIDENCE OF NEOPLASTIC &NON NEOPLASTIC LESIONS

NON NEOPLASTIC	NEOPLASTIC BENIGN	NEOPLASTIC MALIGNANT	TOTAL
28	41	23	92
30.44%	44.56%	25%	100%

Incidence of benign tumor constitutes 44.5% and malignant tumors constitutes 25% of total salivary gland lesions. Incidence of non neoplastic lesions were 30.44% of total salivary gland lesions[table -3],[chart-3].

Table 4:

AGE DISTRIBUTION OF SALIVARY GLAND LESIONS

AGE IN YEARS	BENIGN	MALIGNANT	NON NEOPLASTIC	TOTAL	PERCENTAGE
1-10	1	1	2	4	4.35%
11-20	1	-	7	8	8.69%
21-30	6	1	7	14	15.22%
31-40	10	5	3	18	19.56%
41-50	12	5	5	22	23.92%
51-60	4	5	1	10	10.86%
61-70	6	4	3	13	14.14%
71-80	1	2	-	3	3.26%
Total	41	23	28	92	

Among the neoplastic lesions, tumors were presented over a wide range of 20 to 80 years of age. Of 64 neoplastic lesions, the peak incidence for benign tumors were 30-50 years. Malignant tumors shows a wide range from 30-70 years of age. The mean age for non neoplastic lesions was 33.03 years, benign tumor was 43.39yrs, malignant tumor was 49.95 yrs. In this study the youngest patient was 7 years and the eldest was 80 years of age as in the table 4[chart-4].

TABLE 5:

SEX DISTRIBUTION OF SALIVARY GLAND LESIONS

SEX	BENIGN	MALIGNANT	NON NEOPLASTIC	TOTAL	PERCENTAGE OF TOTAL
MALE	11	8	15	34	36.95%
FEMALE	30	15	13	58	63.05%

Among the 92 cases of total salivary gland lesions 58 cases were female which constitutes 63.05% and 34 cases were male which accounts for 36.94% of salivary gland lesions. Female predominance was observed in all types of neoplastic lesions(58 in female and 34 in male). With male to female ratio of **1 :1.7** [chart-5],[table-5].

TABLE 6

FREQUENCY OF SEX DISTRIBUTION OF SALIVARY GLAND TUMORS

SEX	BENIGN	MALIGNANT	TOTAL	PERCENTAGE
FEMALE	30	15	45	70.32%
MALE	11	8	19	29.68%

Among the neoplastic lesions 45 cases were female(70.32%) and 19cases were male(29.68%) with male to female ratio of 1:2.36 The age distribution of female was 7-80 years and in male it was 32-70 years of age as shown in table 6.

TABLE 7:

SITE DISTRIBUTION OF SALIVARY GLAND LESIONS

SITE	NONNEO PLASTIC	BENIGN	MALIGNANT	TOTAL	% OF TOTAL
PAROTID	9	37	12	58	63.04%
SUBMANDIBULAR	5	-	3	8	8.70%
MINOR SALIVARYGLAND	15	6	5	26	28.26%

Among 92 Cases of salivary gland lesions ,58 cases were seen in parotid with the incidence of 63.04%, 26 cases were seen in minor salivary gland with the incidence of 28.26% and 8 cases in submandibular gland with the incidence of 8.70%[table-7],[chart-6].

TABLE 8:

SITE DISTRIBUTION OF SALIVARY GLAND TUMORS

SITE	**BENIGN**	**MALIGNANT**	**TOTAL**	**%OF TOTAL**
PAROTID	37	12	49	76.56%
SUBMANDIBULAR GLAND	-	3	3	4.68%
MINOR SALIVARY GLAND	6	6	12	18.76%

Of the 64 cases of neoplastic lesions 49 cases were seen in parotid(76.56%), 12 cases (18.76%) seen in minor salivary gland and 3cases (4.68%) were seen in submandibular gland [table-8],[Chart-7].

Of the 49 cases in the parotid gland, Pleomorphic adenoma is the most common which constitutes for 42.18%, followed by Mucoepidermoid carcinoma which constitutes 18.75%. In minor salivary gland Adenoid cystic carcinoma was the most common accounting for 3.12% of all cases.

TABLE-9:

SITE DISTRIBUTION OF BENIGN LESIONS

BENIGN LESIONS	PAROTID	%	SUB MANDIBULAR	MINOR SALIVARY GLAND	%
PLEOMORPHIC ADENOMA	27	42.18%	-	3	4.68%
BASAL CELL ADENOMA	4	6.25%	-	2	3.12%
WARTHIN'S TUMOR	4	6.25%	-	-	-
MYOEPITHELIOMA	1	1.56%	-	-	-

Among the benign lesions of parotid, Pleomorphic adenoma is the most common accounting for 42.18% followed by Basal cell adenoma and Warthins which accounts for (6.25%)[table-9],[chart-8].

TABLE 10:

SEX DISTRIBUTION OF MALIGNANT LESIONS

MALIGNANT TUMOR	FEMALE	MALE	TOTAL	%OF TOTAL
MUCOEPIDERMOID CARCINOMA	9	3	12	52.17%
ADENOID CYSTIC CARCINOMA	2	3	5	21.73%
SALIVARY DUCT CARCINOMA	1	1	2	8.70%
CARCINOMA EX PA	-	2	2	8.70%
BASAL CELL ADENOCARCINOMA	1	-	1	4.35%
PRIMARY SQUAMOUS CELL CARCINOMA	1	-	1	4.35%

Of the 23 malignant lesions, Mucoepidermoid was the most common lesion involving the parotid(52.17%) followed by Adenoid cystic carcinoma (21.73%) as in table 10. The incidence of malignant tumor shows a female preponderance with 9 cases (39.13%) of mucoepidermoid [table-10],[chart-9].

TABLE 11:

SITE DISTRIBUTION OF MALIGNANT SALIVARY GLAND TUMORS

MALIGNANT TUMOR	PAROTID	%	SUB MANDI BULAR	%	MINOR SAL GLAND	%
MUCO EPIDERMOID CA	12	18.75%	-	-	-	
ADENOID CYSTIC CA	1	1.56%	2	3.12%	2	3.12%
CA EX PLEOMORPHIC ADENOMA	1	1.56%	-	-	1	1.56%
SALIVARY DUCT CARCINOMA	-		1	1.56%	1	1.56%
BASAL CELL ADENOCARCIN OMA	-		-	-	1	1.56%
PRIMARY SQUAMOUS CELL CARCINOMA.	1	1.56%	-	-	-	-

Among the salivary gland tumors, malignant lesions occur most commonly in parotid accounting for 15 cases [23.43%] followed by minor salivary gland 5cases [7.81%] and submandibular gland 3cases (4.68%) [table-11], [chart-10].

TABLE 12:

FREQUENCY OF NON NEOPLASTIC LESIONS

NON NEOPLASTIC LESIONS	MALE	FEMALE	TOTAL	%OF TOTAL
CHRONIC SIALADENITIS	5	3	8	28.57%
MUCOUS RETENTION CYST	6	8	14	50%
KIMURA'S DISEASE	1	-	1	3.57%
KUTTNER TUMOR	1	-	1	3.57%
BENIGN CYSTS	2	1	3	14.29%
LYMPHOEPITHELIAL CYST	-	1	1	3.57%

Non neoplastic lesions are commonly seen in the age group of 20-40 years of age.Male preponderance was seen in these lesions with 15 cases of male and 13 cases of female. [table 12],[chart-11]

TABLE 13:

SITE DISTRIBUTION OF NON NEOPLASTIC LESIONS

NON NEOPLASTIC	PAROTID	%	SUB MANDI BULAR	%	MINOR SALIVARY	%
CHRONIC SIALADENITIS	3	10.72%	5	17.85%	0	0
MUCOUS RETENTION CYST	0	0	0	0	14	50%
KIMURA'S DISEASE	1	3.57%	0	0	0	0
KUTTNER TUMOR	0	0	1	3.57%	0	0
LYMPHOEPITH ELIAL CYST	1	3.57%	0	0	0	0
BENIGN PAROTID CYST	3	10.72%	0	0	0	0

Among the non neoplastic lesions, Mucous retention cyst were the most common. It accounts for 14 cases (50%) in minor salivary gland, followed by chronic sialadenits in submandibular gland. One case each of Kimura's and Kuttner tumor were reported in this study. [table-13],[chart-12]

TABLE 14:

INCIDENCE OF ALL SALIVARY GLAND TUMORS

LESIONS	NUMBER	PERCENTAGE
PLEOMORPHIC ADENOMA	30	46.87%
BASAL CELL ADENOMA	6	9.37%
WARTHIN'STUMOR	4	6.25%
MYOEPITHELIOMA	1	1.56%
MUCOEPIDERMOID CA	12	18.75%
ADENOID CYSTIC CARCINOMA	5	7.81%
SALIVARY DUCT CARCINOMA	2	3.13%
CARCINOMA EX PLEOMORPHIC ADENOMA	2	3.13%
BASAL CELL ADENOCARCINOMA	1	1.56%
PRIMARY SQUAMOUS CELL CARCINOMA	1	1.56%

The benign tumor most common in this study was pleomorphic adenoma[fig 1,2] which accounts for 30cases. The incidence was 46.87% cases of total neoplasm and 73.17% of benign salivary gland tumors. Basal cell adenoma[[figure11,12,13] is the second most common benign tumor accounting for 9. 37% of all neoplastic lesions and 14.63% of benign tumors. Warthin's tumor[figure14,15] accounts for 6 .25% cases of all neoplastic lesions and 9.76% of benign tumors. Myoepithelioma[figure 16] account for 1.56% of neoplastic lesion and 2.44% of benign lesions.

Pleomorphic adenoma shows a peak incidence in 3rd- 5th decade of life with mean age of 43.97 yrs with female preponderance (58.54%). Of the 30 cases, 27 arise from parotid and 3 cases from submandibular gland. Out of 6 cases of basal cell adenoma 4 cases arise in the Parotid and 2 cases from minor salivary glands. Out of 4 cases of warthin's tumor, all cases arise in parotid with a male preponderance. The mean age was 61 yrs.

Among the malignant tumors, Mucoepidermoid [figure 17-23] was the commonest accounting for 18.75% of all neoplasms and 52.17% of malignant tumors. The second most common tumor was Adenoid cystic carcinoma [figure24,25] which constitutes about 7.81% of all neoplasms and 21.73% of malignant tumors. Salivary duct carcinoma[figure 27], carcinoma ex pleomorphic adenoma 2 case each accounting for 3.13% followed by Basal cell adenocarcinoma[figure 26],primary squamous cell carcinoma[figure 28] one case of each accounting for 1.56% of all neoplasms [table-14],[chart-13].

Mucoepidermoid carcinoma shows a female preponderance[75%] with mean age of 42.5yrs. Of the 12 cases all arise from parotid gland[18.75%]. The youngest patient reported was 7 years old female child.

Adenoid cystic carcinoma shows a male preponderance [60%]. Out of 5 cases one occur in parotid[1.56%],2 cases each in submandibular and minor salivary gland accounting for 3.12% of malignant tumors.

Of the 28 non neoplastic lesions, 14 were mucous retention cyst [figure 29],8 were chronic sialadenitis [figure30]. Non neoplastic lesions shows peak incidence in 2nd to 3rd decade of life. Among 28 cases 8 cases occur in the parotid,7 cases occur submandibular gland. Remaining 14 cases occur in minor salivary gland.[chart-12]

FREQUENCY OF EXPRESSION OF p63 AND OTHER MARKERS IN SALIVARY GLAND TUMORS

TABLE:15 p63 EXPRESSION IN PLEOMORPHIC ADENOMA

PLEOMORPHIC ADENOMA	**p63[ABLUMINAL CELLS]**	**CK-7[LUMINAL CELLS]**
CASES-1	POSITIVE	POSITIVE
CASE-2	POSITIVE	POSITIVE
CASE-3	POSITIVE	POSITIVE
CASE-4	POSITIVE	POSITIVE

In our study, p63 myoepithelial marker[figure35,36] was studied in 4 cases of pleomorphic adenoma and all the abluminal cells [myoepithelial cells]were positive for p63. All luminal cells were positive for CK-7[figure37]. Expression of myoepithelial cell marker p63 in Pleomorphic adenoma have confirmed the role of myoepithelial cells in histiogenesis of this tumors.[64] Immunohistochemical positivity of myoepithelial cell marker in Pleomorphic adenoma indicates the origin of this tumor, is from **intercalated duct of salivary gland.**

TABLE 16: p63 EXPRESSION IN MUCOEPIDERMOID CARCINOMA

MUCOEPIDERMOID CARCINOMA	p63[INTERMEDIATE CELLS]	CK-7[MUCINOUS CELLS]	Ki-67
LOW GRADE	POSITIVE	POSITIVE	<3%
INTERMEDIATE GRADE	POSITIVE	POSITIVE	<10%
HIGH GRADE	POSITIVE	POSITIVE	-

In mucoepidermoid carcinoma , the intermediate cells shows positive for CK-7, p63 [figure44] and mucocytes shows positive for CK-7[figure43]. Limited or lack of myoepithelial cells in mucoepidermoid carcinoma [figure44] indicate the minimal myoepithelial differentiation in hisitogenesis of mucoepidermoid carcinoma.

The absence of myoepithelial cells in excretory or striated ducts of salivary gland and negative staining for myoepithelial markers in MEC suggest that it originates from **excretory or striated duct component** of salivary glands.

TABLE:17

IHC MARKERS IN OTHER BENIGN TUMORS:

TUMORS	p63[ABLUMINAL CELLS]	CK7[LUMINAL CELLS]	Ki-67 INDEX
BASAL CELL ADENOMA	POSITIVE	POSITIVE	> 3%
WARTHINS TUMOR	POSITIVE[BASAL CELLS]	POSITIVE	3%
MYOEPITHELIOMA	POSITIVE	NEGATIVE	-

In basal cell adenoma the luminal cells are positive for CK-7 [figure39],the basaloid cells are positive for p63[figure-40]. While the luminal secretory material is positive for PAS by histochemistry [figure-12].

In myoepithelioma [figure48] the myoepithelial cells have taken the p63 ,while in Warthin's tumor the luminal cells shows CK-7 positive[figure 41] and the basal cells shows p63 positive[figure 42].

TABLE 18 :MARKERS IN OTHER MALIGNANT TUMORS

ADENOID CYSTIC CARCINOMA	CK-7 POSITIVE [LUMINAL CELLS]	CD-117 POSITIVE [LUMINAL CELL]
BASAL CELL ADENOCARCINOMA	Ki-67 >50%	POSITIVE
SALIVARY DUCT CARCINOMA	HER2/NEU	MEMBRANE POSITIVE[2+]
PRIMARY SQUAMOUS CELL CARCINOMA	CK-7	NEGATIVE
	CK-20	NEGATIVE

In adenoid cystic carcinoma the luminal cells are positive for CK-7[figure-49]]and CD-117[figure-50]. The surrounding myoepithelial cell/basal cells are positive for p63.

As stated by Mino M et al ,[59] CD-117 was previously reported as specific marker for Adenoid cystic carcinoma ,but recently it has been proved to be non specific.[65] However the intensity and extent of CD-117 staining is more in Adcc when compared to other tumors.

BASAL CELL ADENOCARCINOMA: The inner luminal cells were positive for CK-7[figure 51] and Ki-67 index was > 50% in this tumor[figure-52].

SALIVARY DUCT CARCINOMA

Immunohistochemistry done with HER2/neu showed diffuse cytoplasmic positivity[figure53]. About 70-80% of salivary duct carcinoma showed overexpression of HER2/neu and p53, with more than half of the cases having 3+ HER -2 /neu positivity.

PRIMARY SQUAMOUS CELL CARCINOMA :

In our study IHC was done with CK-7 and CK-20, both were found to be negative [figure54,55]. This favors the diagnosis of primary squamous cell carcinoma over mucoepidermoid carcinoma which shows CK-7 positivity.[10.]

Figure 1 Pleomorphic adenoma with Solid glistening grey white areas.

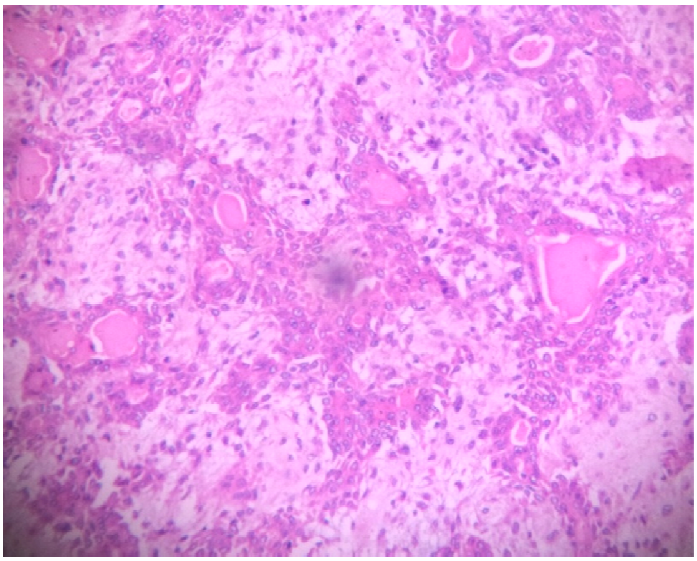

Figure 2.Pleomorphic adenoma,-Ductal structures surrounded by myoepithelial cells H&E (10x)

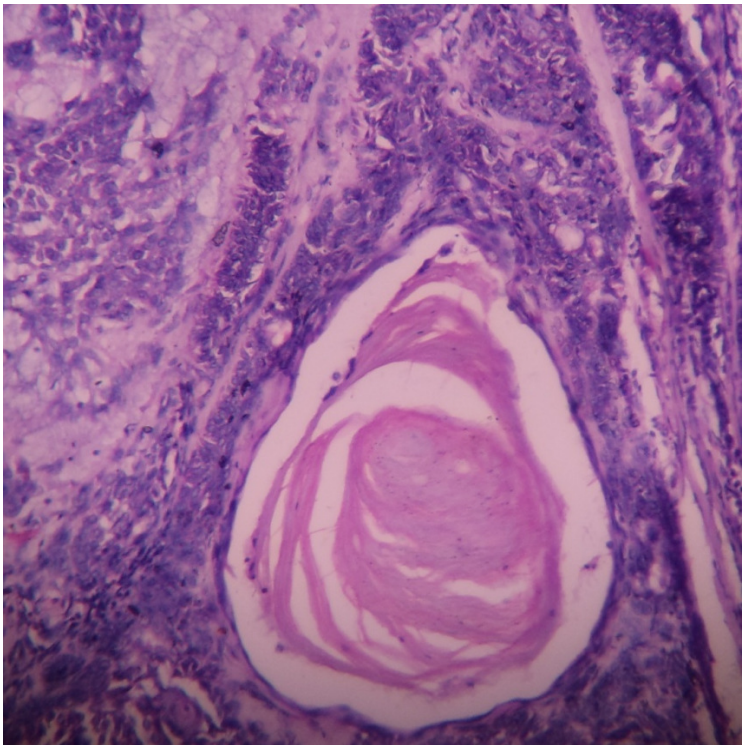

Figure 3.Pleomorphic Adenoma showing squamous metaplasia.H&E(40x).

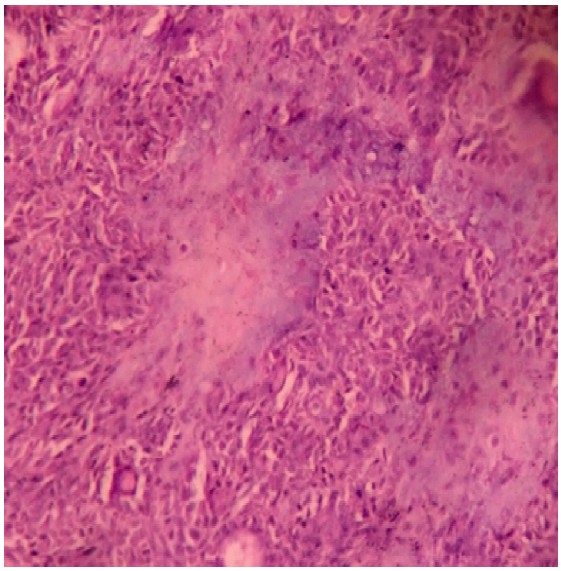

Figure 4.Pleomorphic adenoma with cartilagenous areas. H&E (10x)

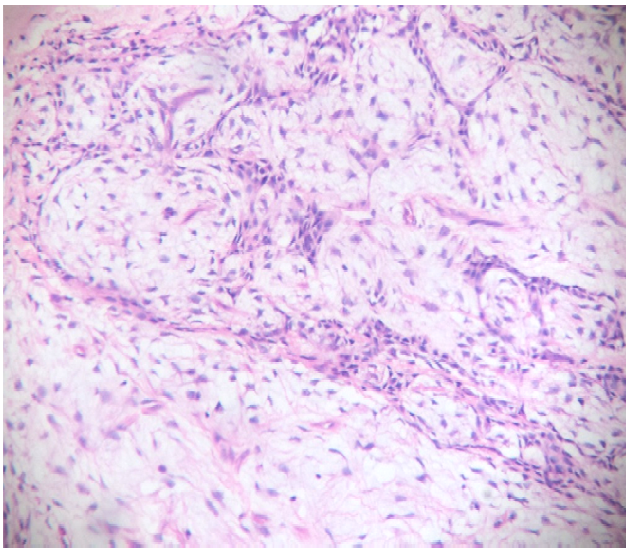

Figure 5. Pleomorphic adenoma showing myxoid stroma with stellate cells.H&E(10x).

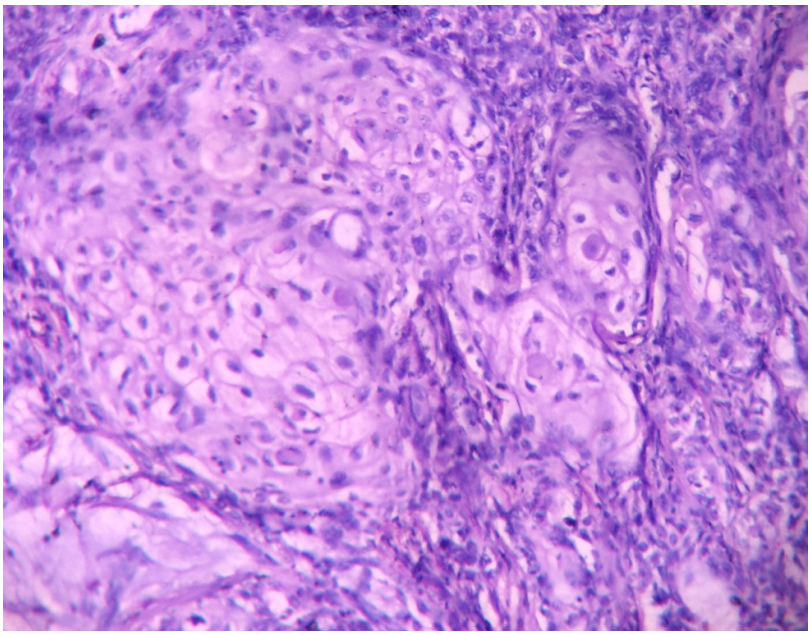

Figure 6.Pleomorphic adeoma with sebaceous differentiation.H&E(40x).

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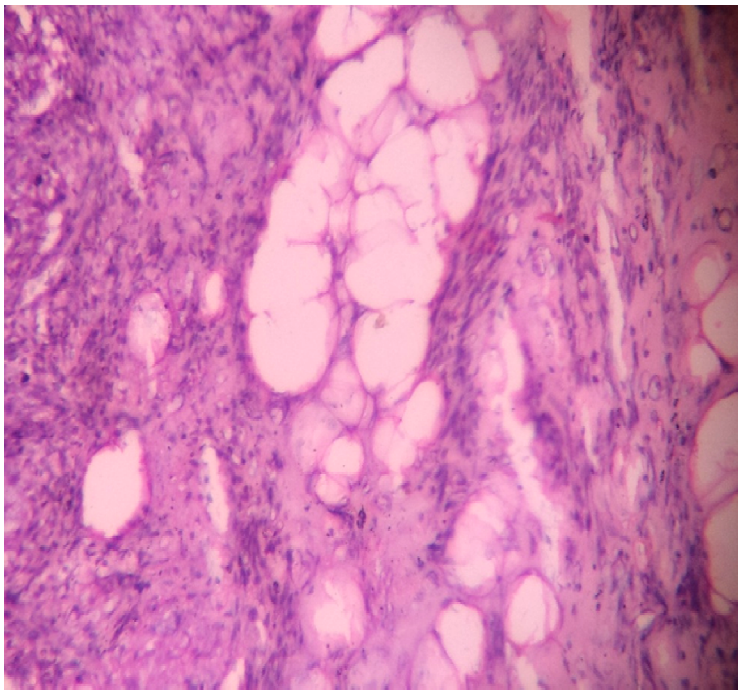


Figure 7.Pleomorphic adenoma with lipomatous areas.H&E(40x)

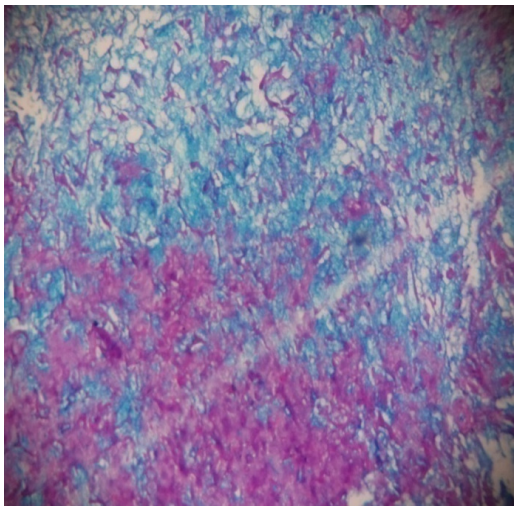


Figure 8. Pleomorphic adenoma-Alcian blue:shows positive chondromyxoid areas. (10x)

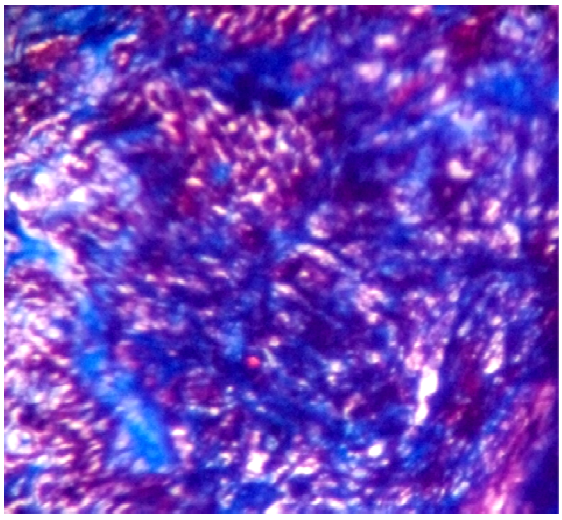


Figure 9.Pleomorphic adenoma- cartilagenous areas shows blue colour with Masson trichrome (40x).

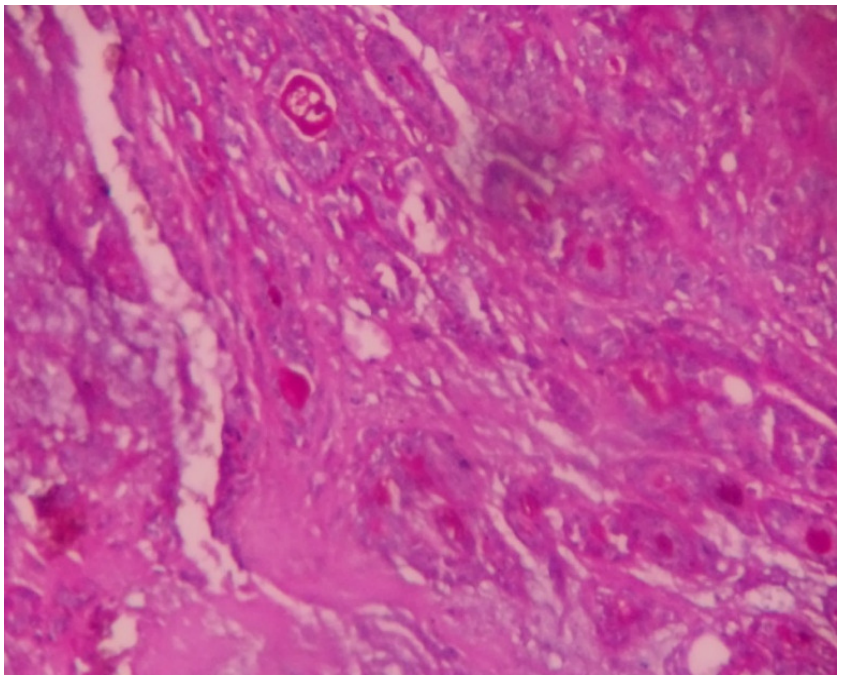


Figure 10.Pleomorphic adenoma showing intraluminal PAS positive secrections(10x).

Figure 11. Gross picture of basal cell adenoma shows a well circumscribed,solid light tan cut surface.

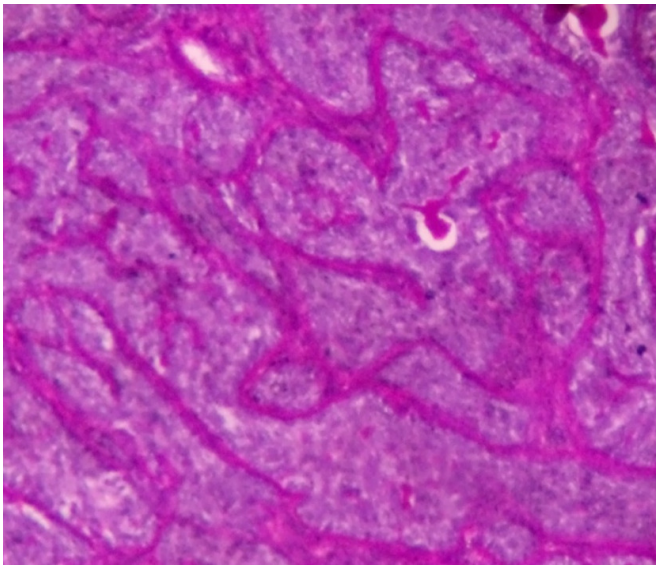


Figure12.Basal cell adenoma shows PAS – positive basement membrane (10x).

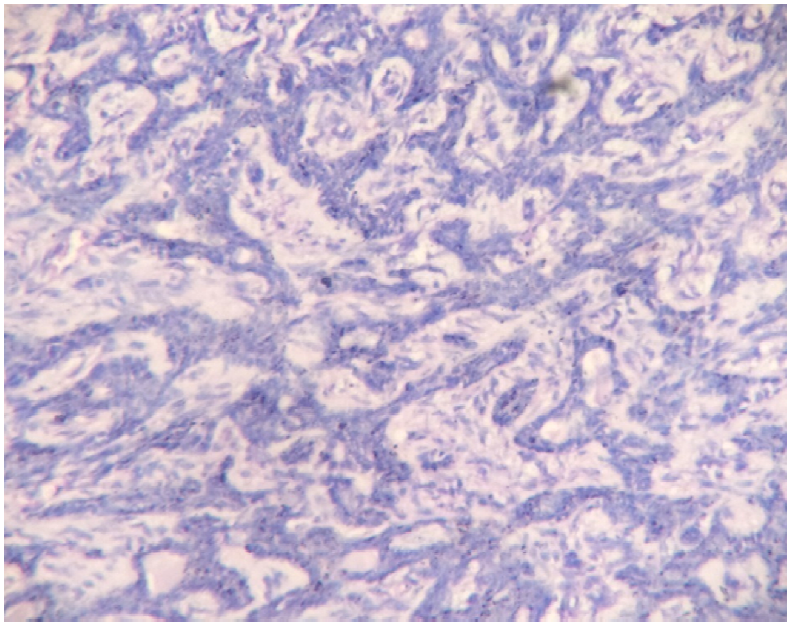
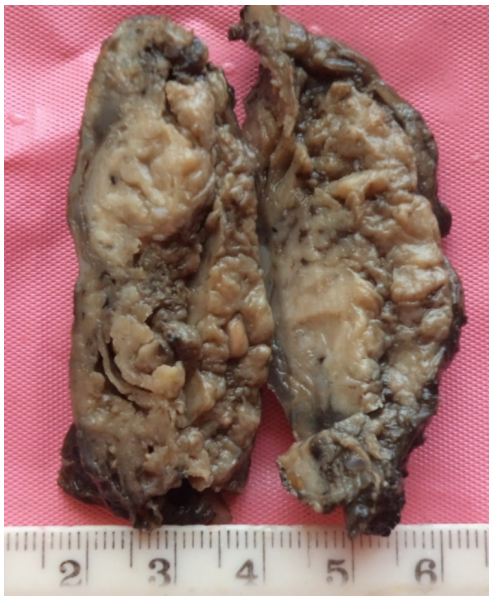


Figure 13.Basal cell adenoma trabecular type;shows cuboidal cell with bland nuclei.(10x).



WARTHINS TUMOR:GROSS[patho no:830/14]

Figure 14.Warthins tumor : shows grey white areas with cystic and papillary projections.

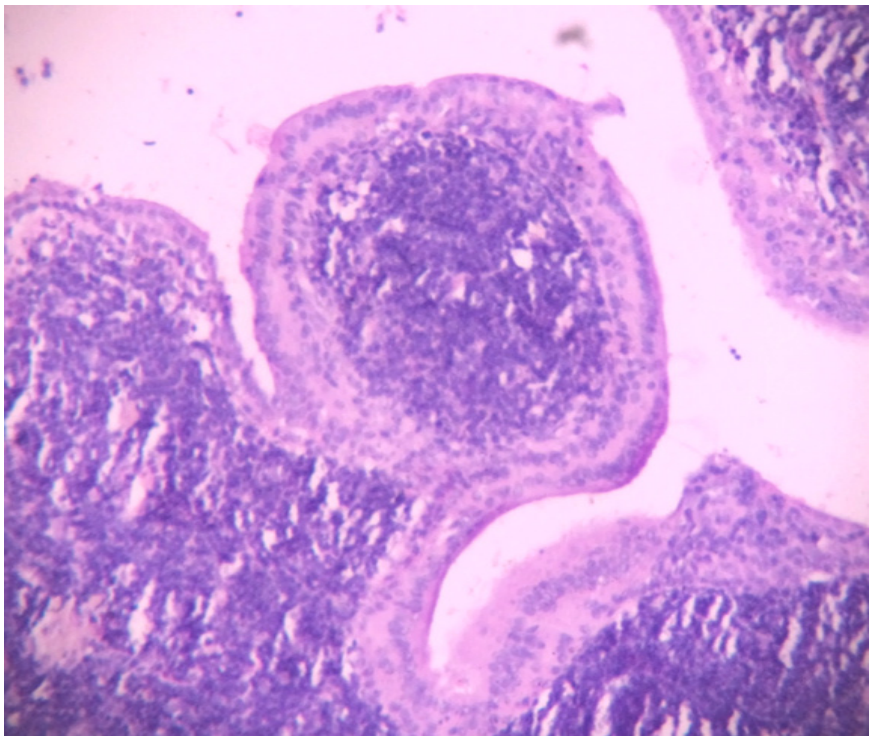


Figure 15.Warthins tumor shows a papillary epithelial lining composed of two layers of epithelial cells with oncocytic features. H&E (10x).

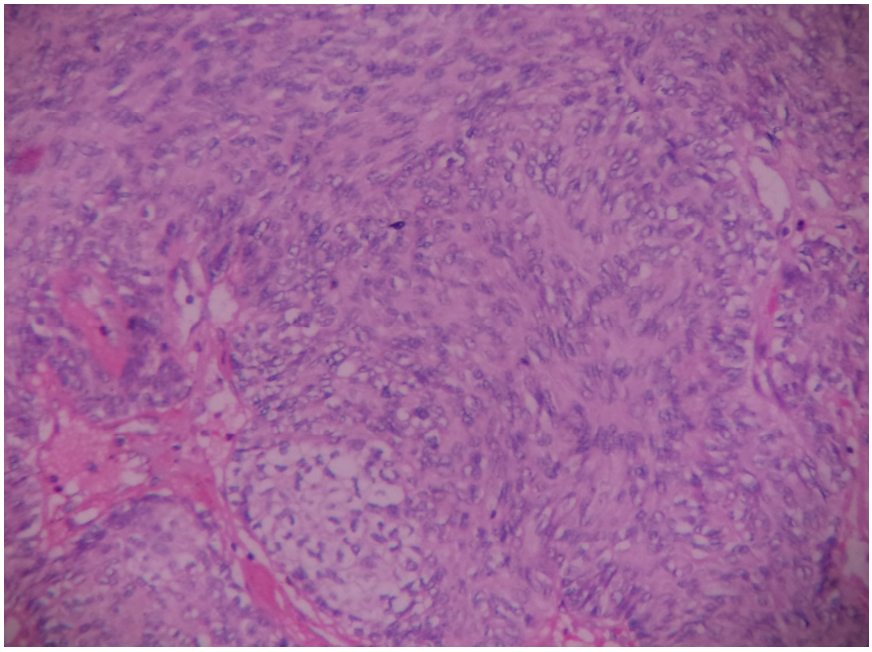


Figure16: Myoepithelioma.showing fascicles of spindle cells with ductal structures.H&E(10x)

Figure 17.Gross picture of Mucoepidermoid Carcinoma -Low grade with cystic areas

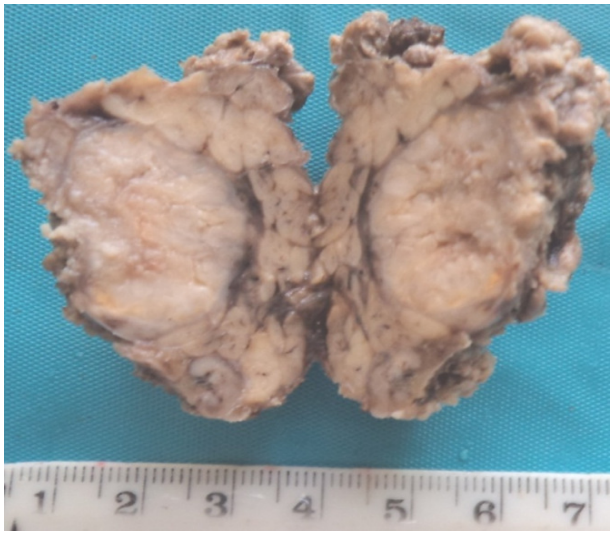


Figure 18.Gross picture of high grade Mucoepidermoid
shows predominantly solid areas.

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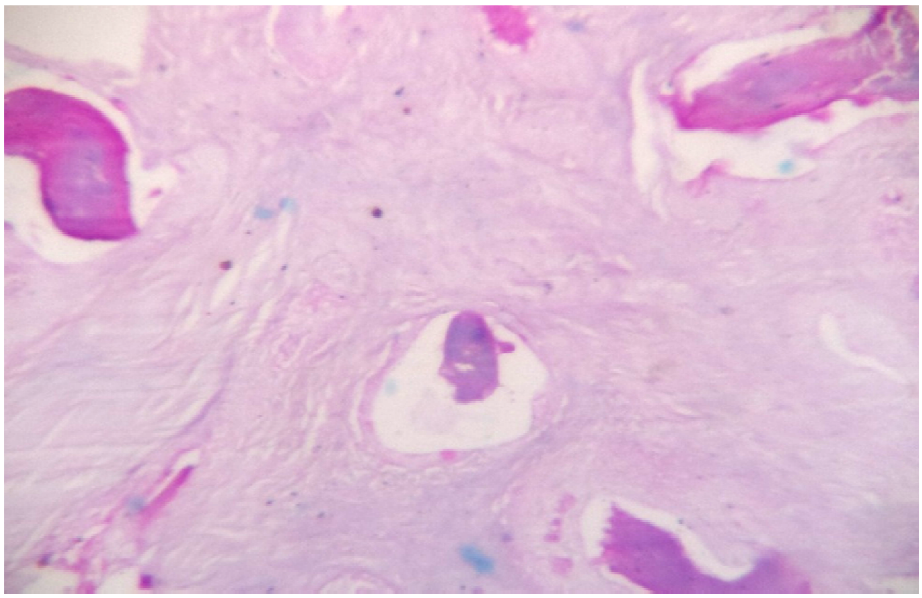

Figure 19. Mucoepidermoid carcinoma low grade shows PAS positive in mucous cells.PAS stain (10x).

.

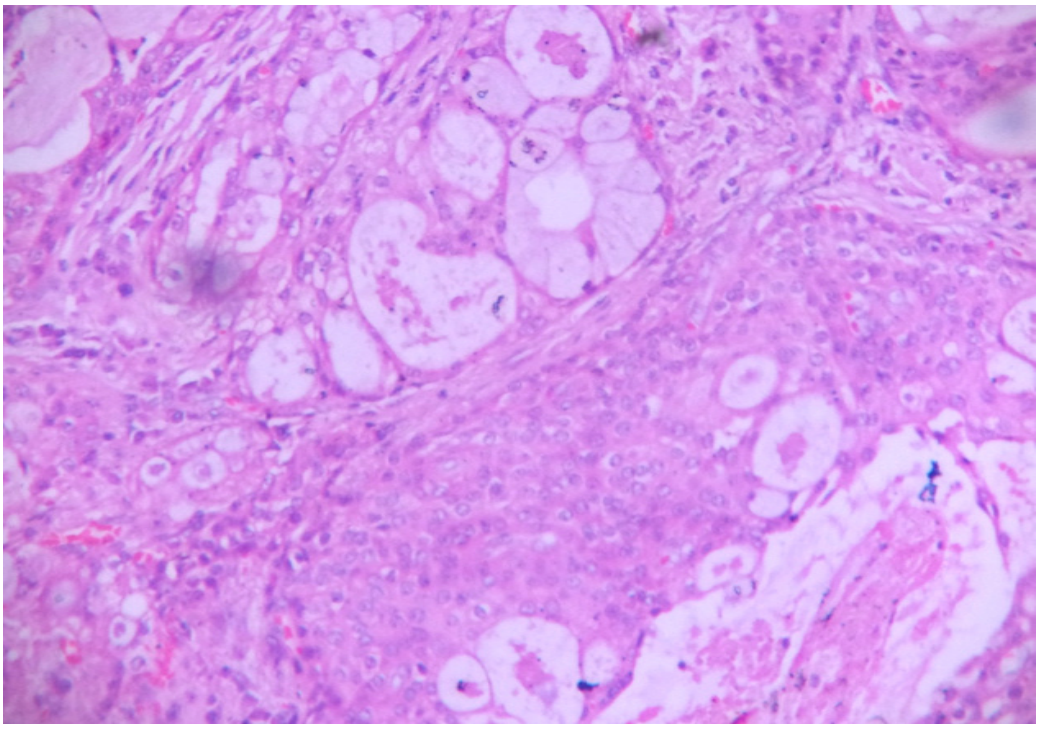

Figure 20. Mucoepidermoid carcinoma low grade shows multiple cyst spaces lined by mucocytes admixed with intermediate cells.H&E (10x).

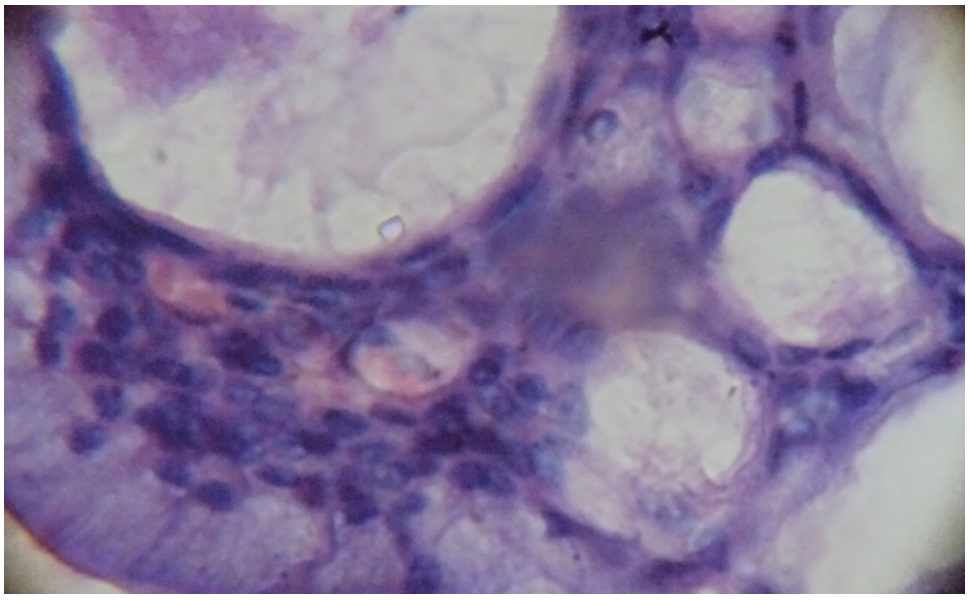

Figure 21.High power of mucocytes in low grade mucoepidermoid carcinoma. H&E (40x).

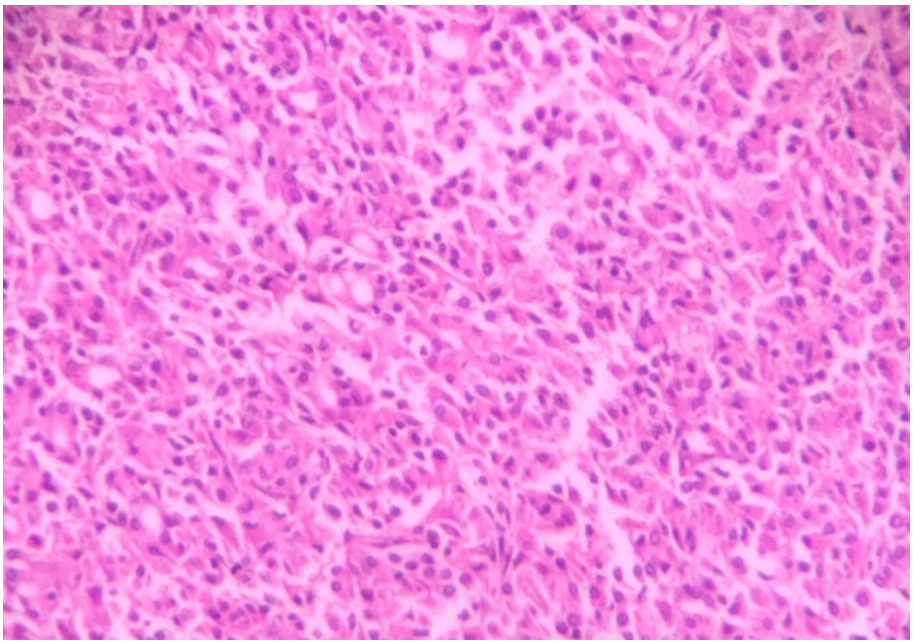

Figure 22.Mucoepidermoid carcinoma intermediate grade.shows predominantly intermediate cells with few mucocytes. H&E (10x).

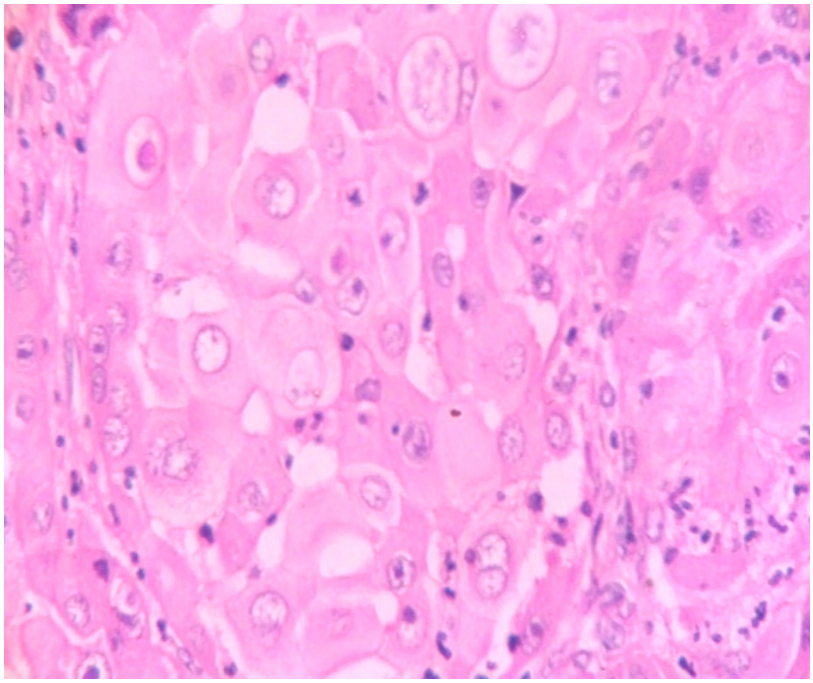

Figure 23. Mucoepidermoid carcinoma high grade shows epidermoid cells with moderate nuclear pleomorphism.H&E (40x).

GROSS PICTURE OF ADENOID CYSTIC CARCINOMA

Figure 24.Gross picture of Adenoid cystic carcinoma firm ,tan with areas of haemorrhage.

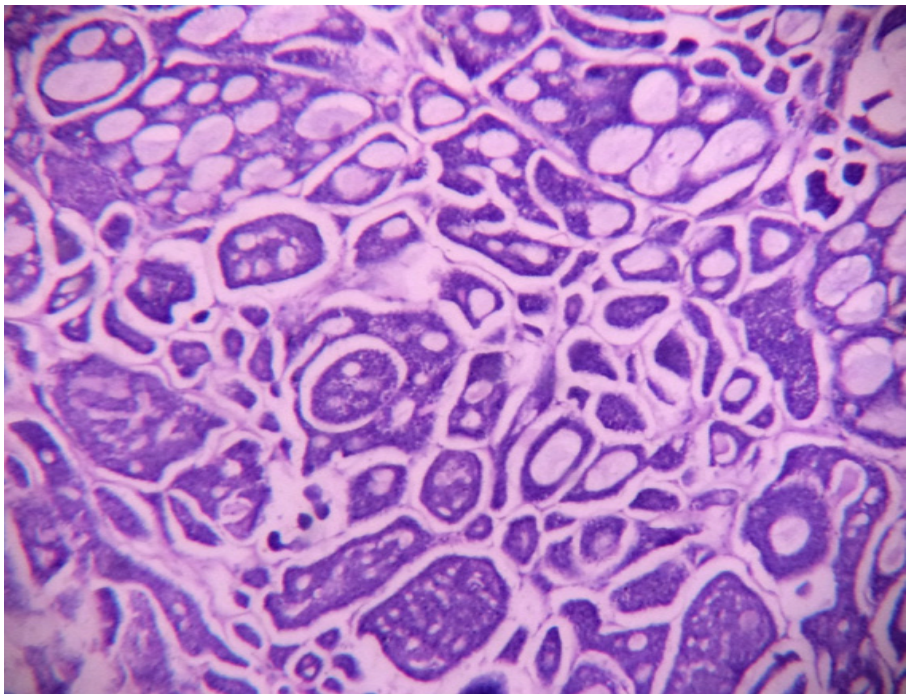

Figure 25:Microscopy of Adenoid cystic carcinoma showing basaloid cells arranged in tubules and cribriform pattern.H&E(10x).

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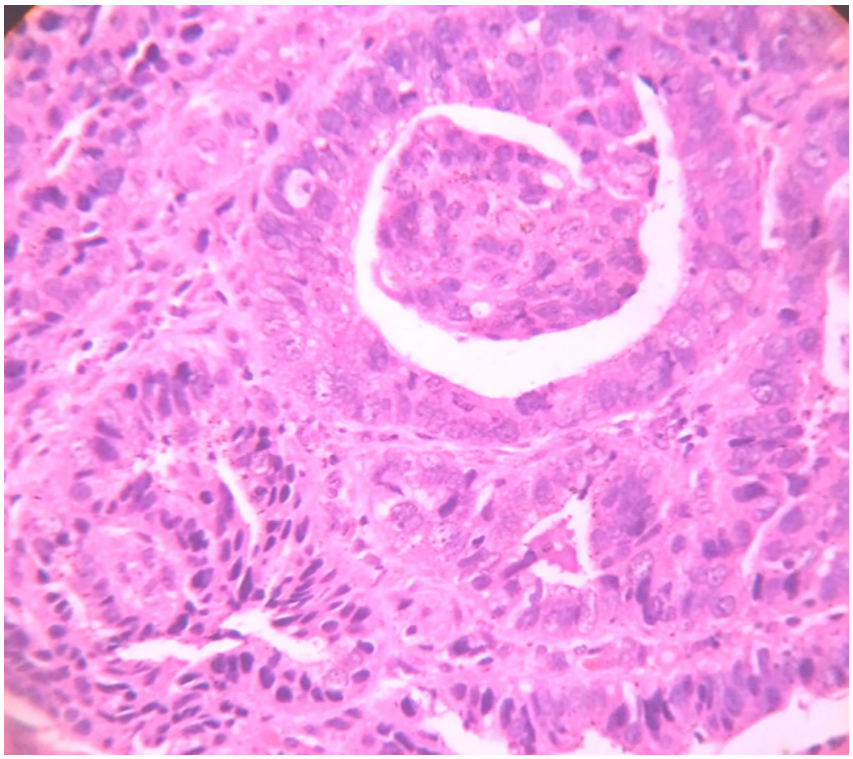

Figure 26.Basal cell adenocarcinoma.microscopy showing basaloid cells with prominent nucleoli.H&E (10x).

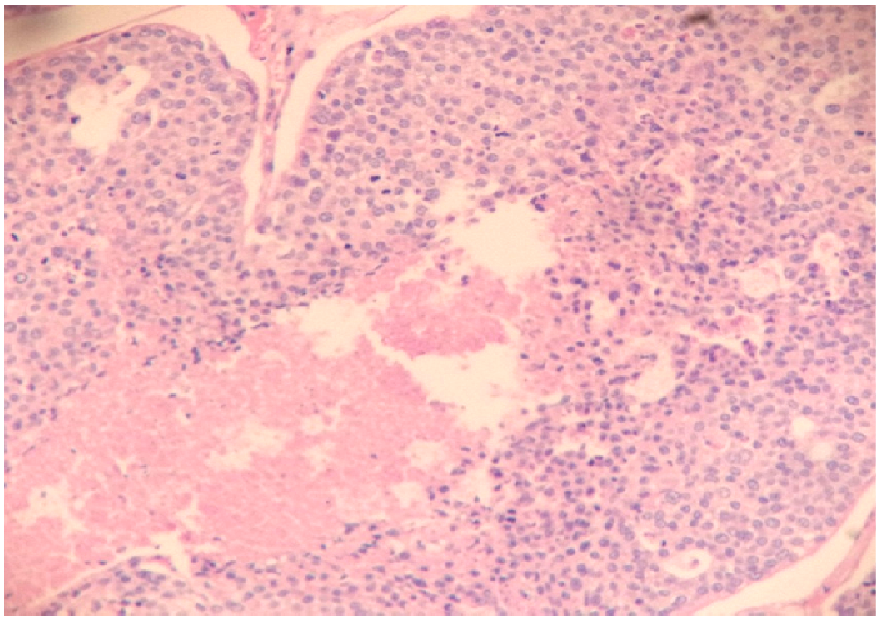

Figure 27.Microscopy of salivary duct carcinoma with central comedo like necrosis. H&E (10x).

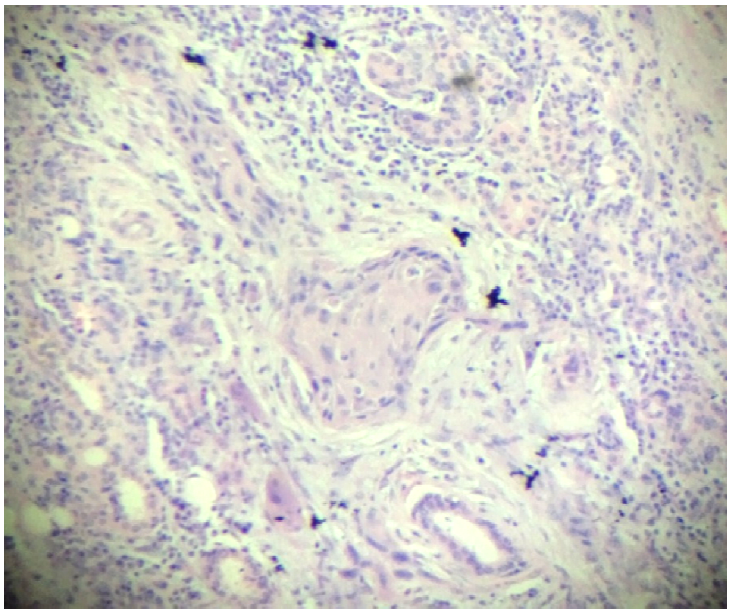

Figure 28.Microscopy of squamous cell carcinoma with prominent keratin pearl.H&E (10x).

NON NEOPLASTIC LESIONS.

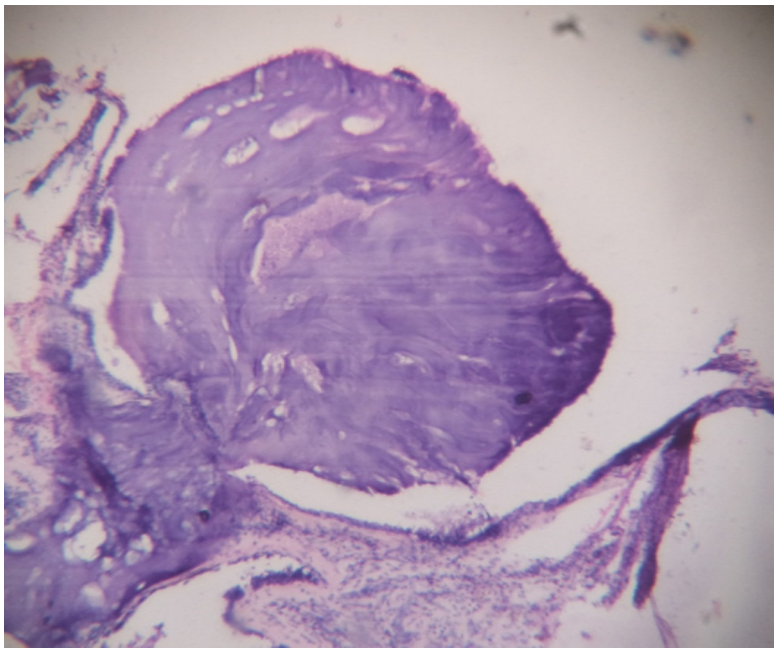

Figure 29.Mucus retention cyst shows cyst containing mucin lined by columnar epithelium.H&E (10x).

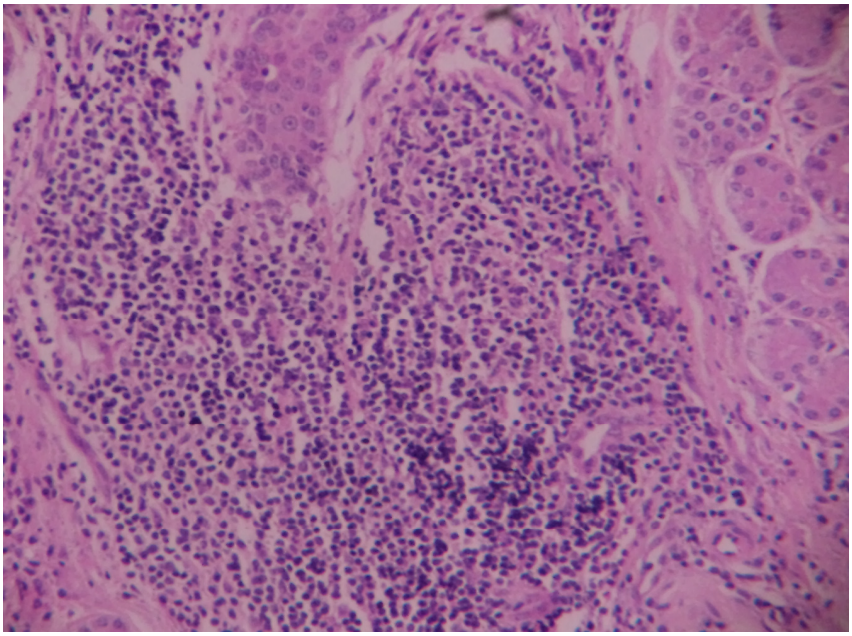

Figure 30. Chronic sialadenitis. Dilated ducts with lymphocytic infiltrate.H&E (10x).

KIMURA'S DISEASE:

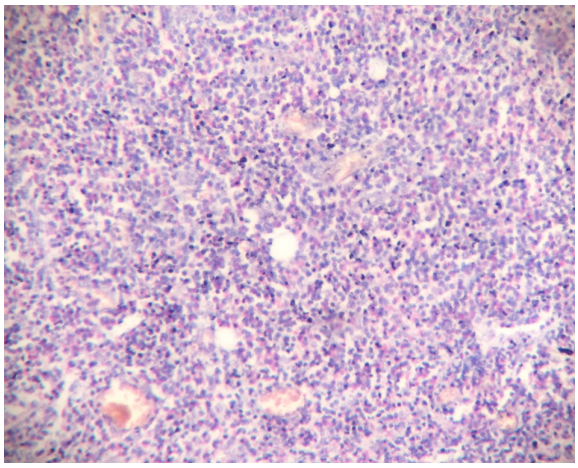

Figure 31 .Kimura disease- .Microscopy shows prominent lymphocytes,eosinophils and vascularization.H&E scanner view .

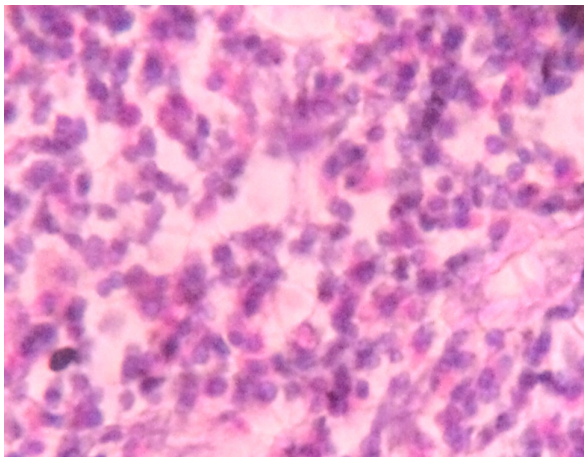

Figure 32. Kimura disease-H&E(40x).

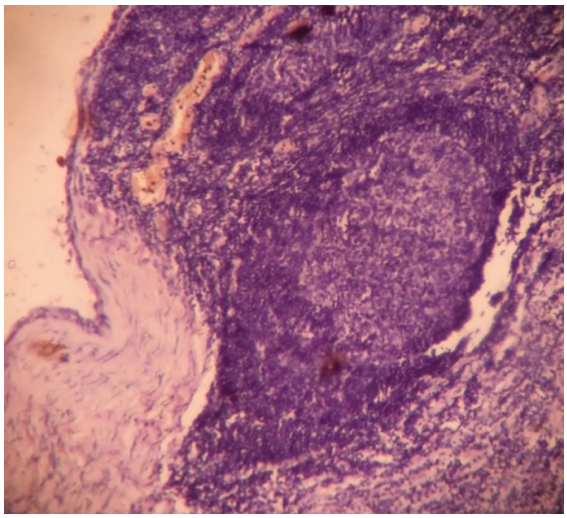

Figure 33.Lymphoepithelial cyst shows undulating luminal surface lined by columnar epithelium with underlying dense lymphoid tissue.H&E(10x).

IMMUNOHISTOCHEMISTRY IN SALIVARY GLAND TUMORS.

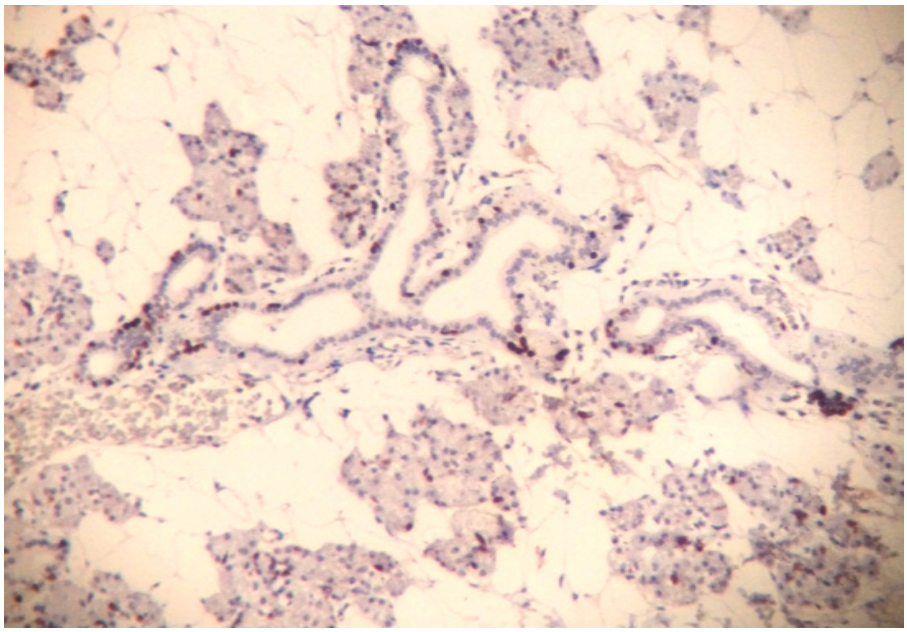

Figure 34a. P63 in nuclei of both myoepithelial and basal cells that surrounds the secretory ductal units. (10x).

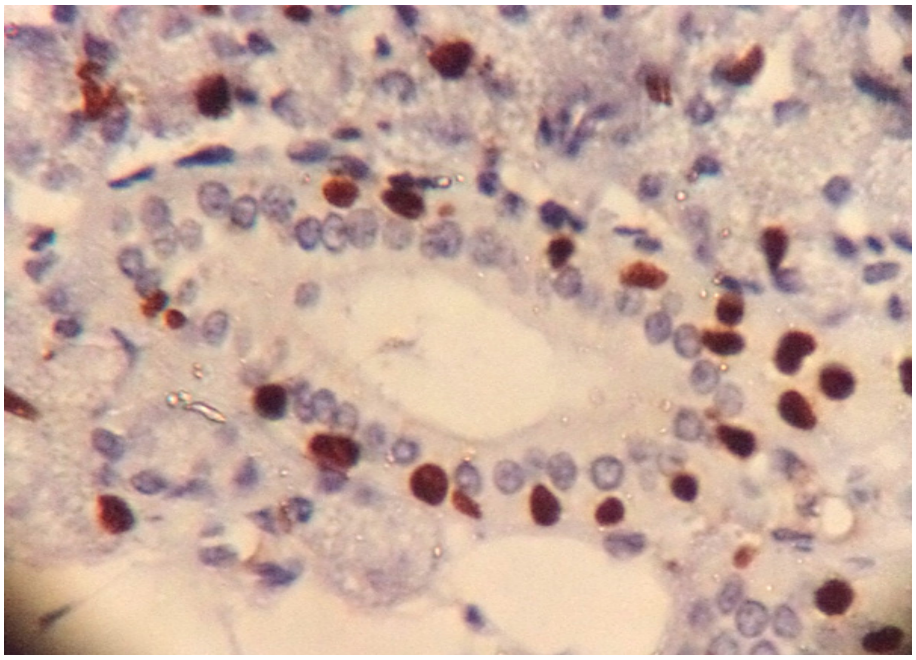

Figure 34b .P 63 in normal intercalated duct- myoepithelial cells are positive ,(40x).

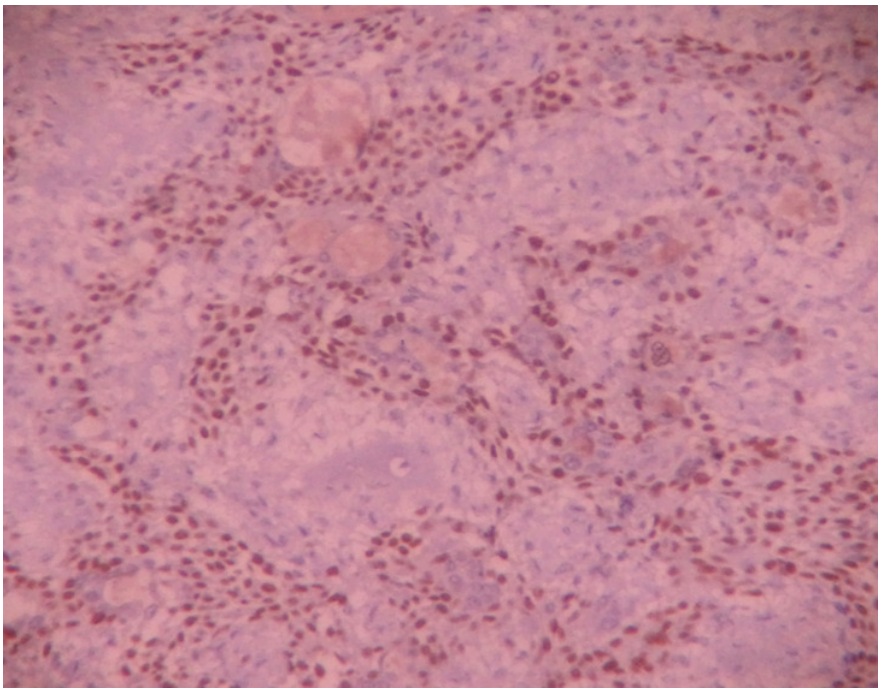

Figure 35. P63 in Plemorphic adenoma myoepithelial cells are positive. (10x).

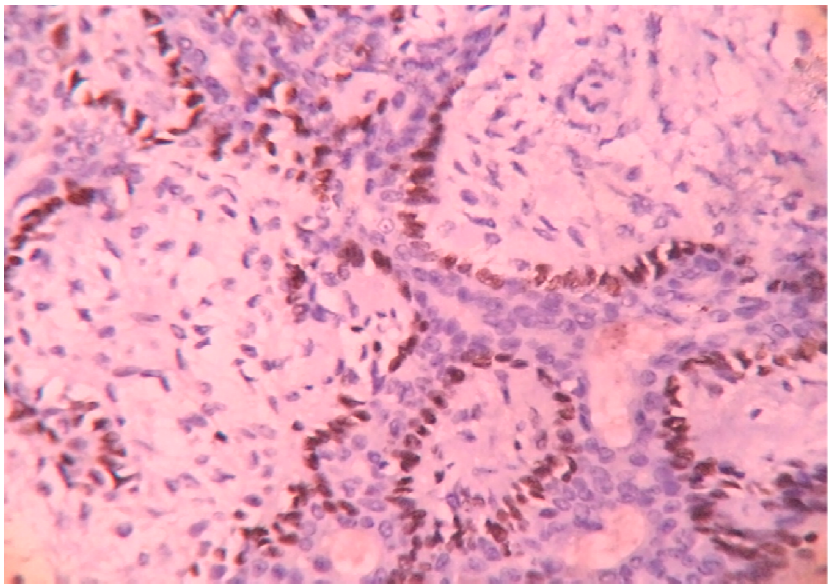

Figure36.P63 in Pleomorphic adenomas- the myoepithelial cells surrounding the luminal cells are positive for P63. (40x).

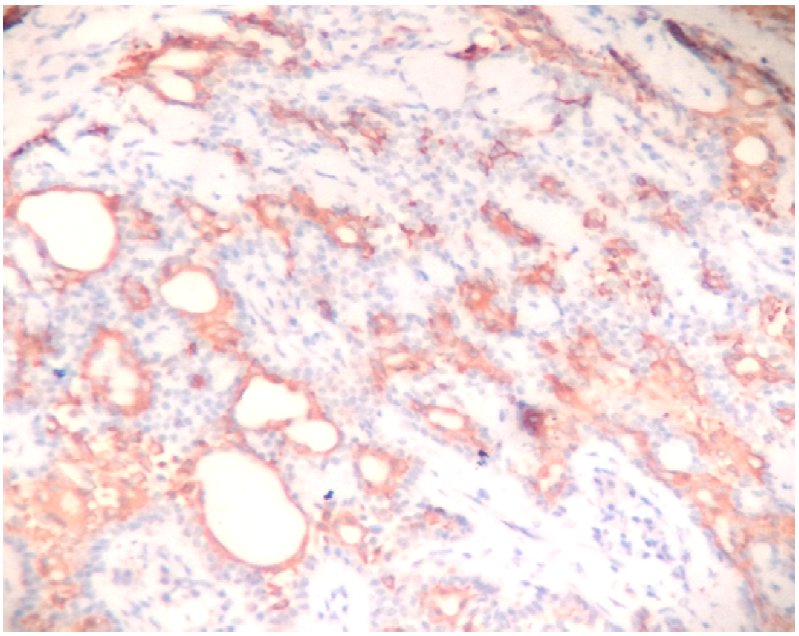

Figure 37.Pleomorphic Adenoma luminal cells are positive for cytokeratin-7 (10x).

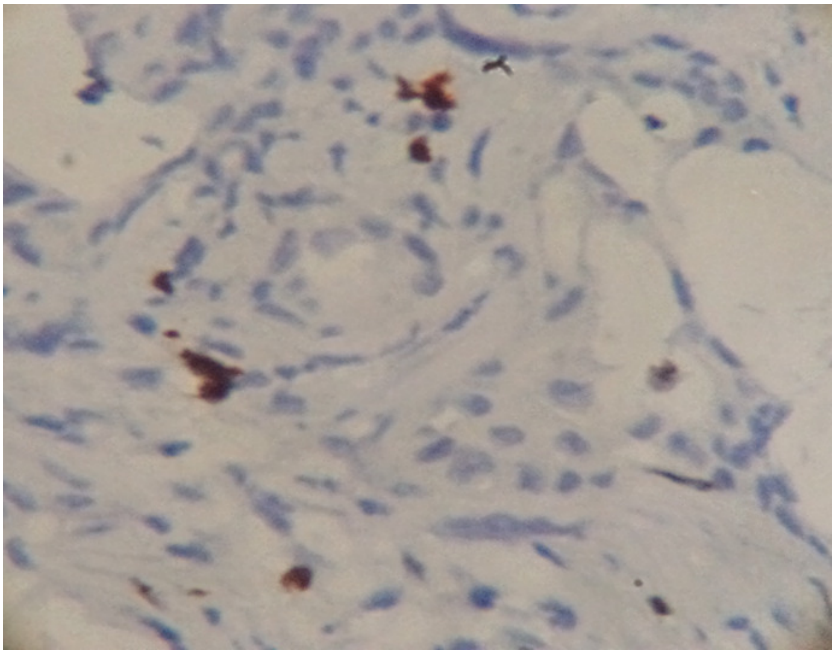

Figure 38.Ki-67 in Pleomorphic adenoma-Ki-67 index < 1%.(40x).

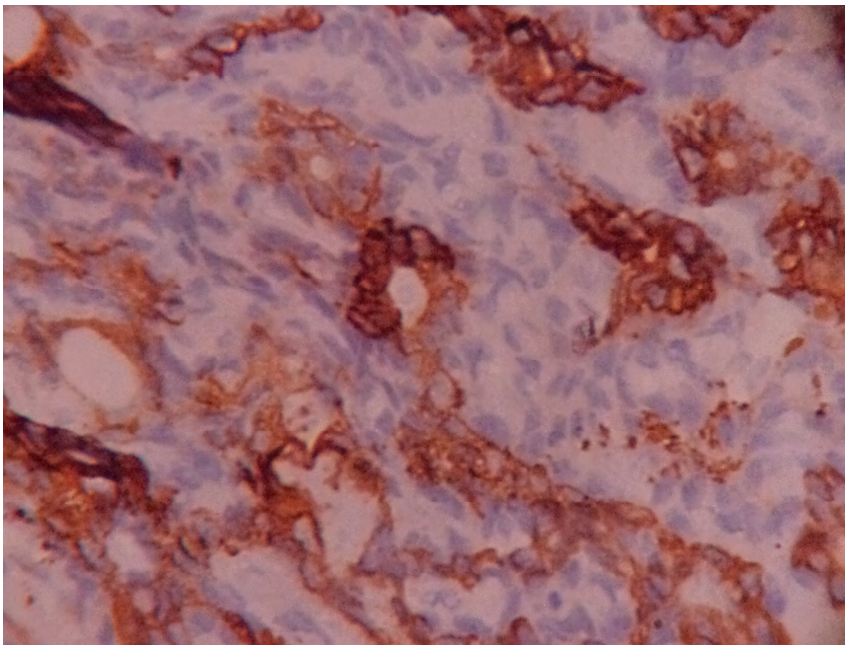

Figure 39.Basal cell Adenoma luminal cells have taken cytokeratin-7 (10x)

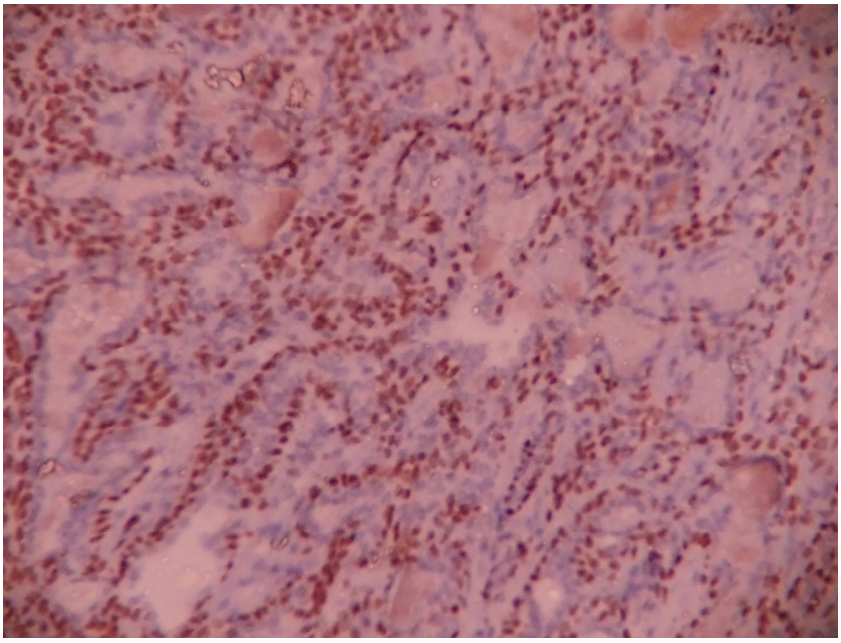

Figure 40 .Basal cell Adenoma.- Myoepithelial cells surrounding the luminal cells have taken P63 10(x).

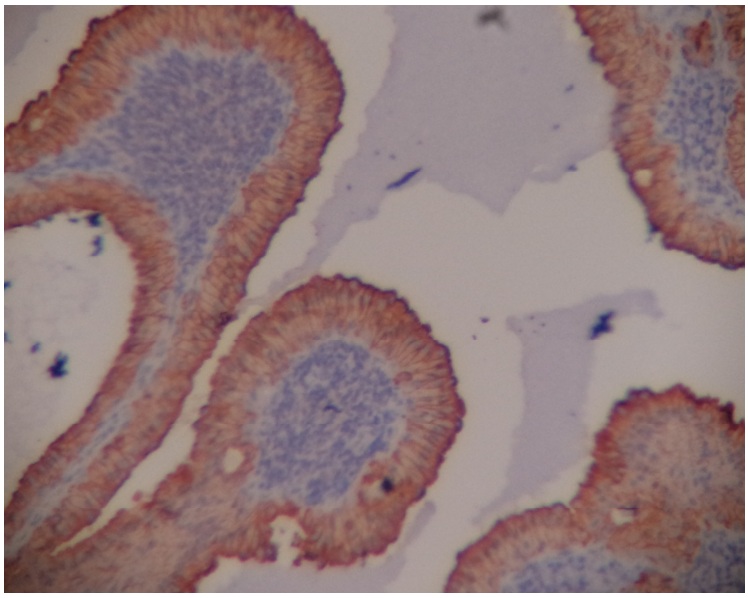

Figure 41.Warthins tumor luminal epithelial cells are positive for cytokeratin-7 (10x).

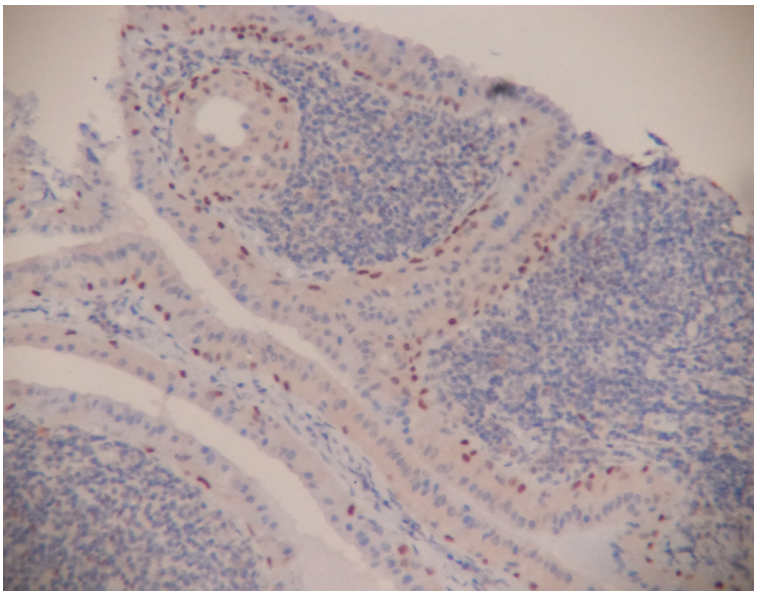

Figure 42.Warthins tumor basal cells are positive for P63(10x).

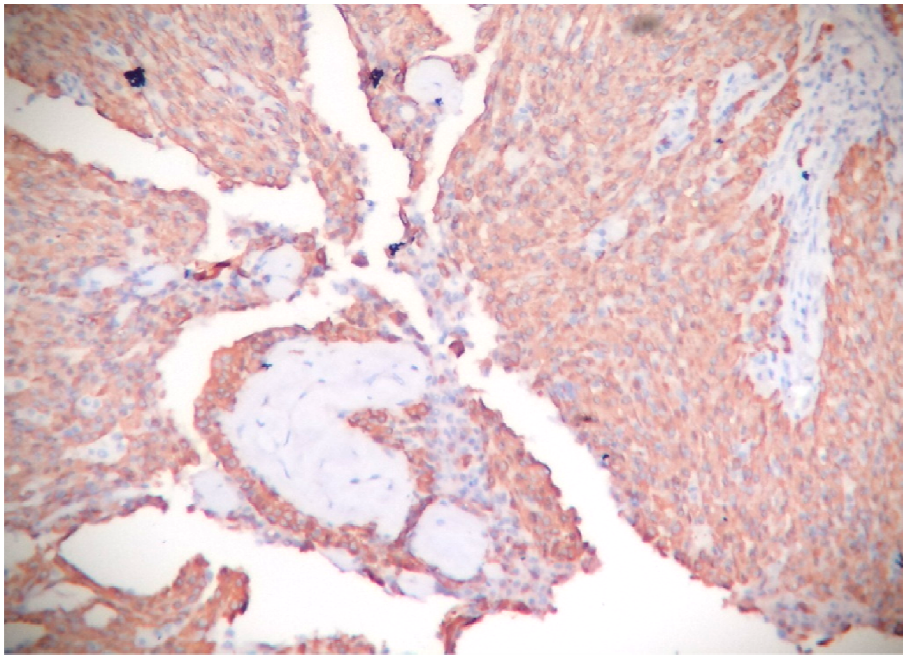

Figure 43. Mucoepidermoid carcinoma. CK-7 has taken up by the mucocytes and intermediate cells(10x).

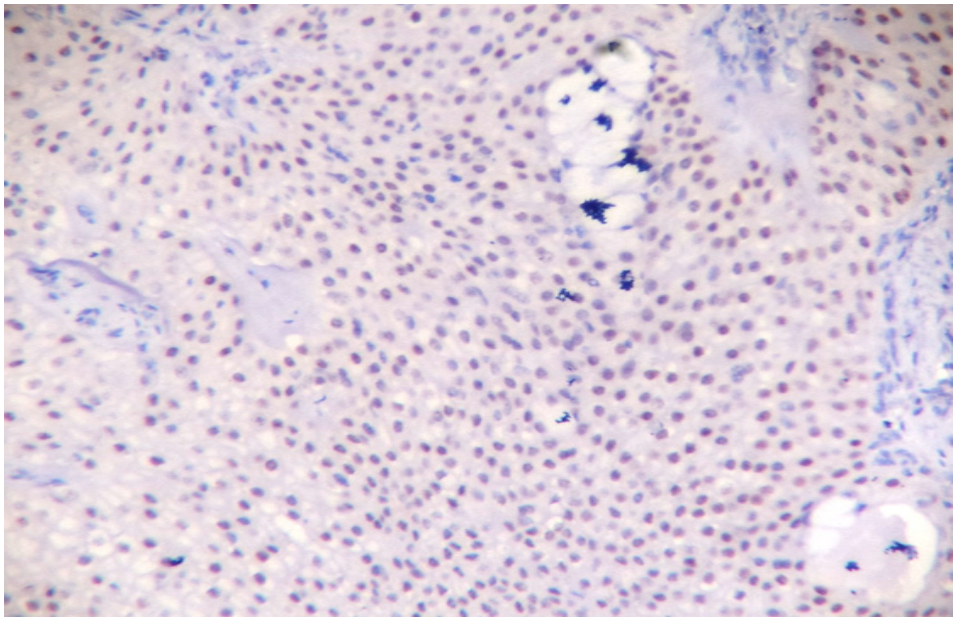

Figure 44. Mucoepidermoid carcinoma. P63 is expressed only in the intermediate epidermoid cells , shows absence of myoepithelial cells (10x).

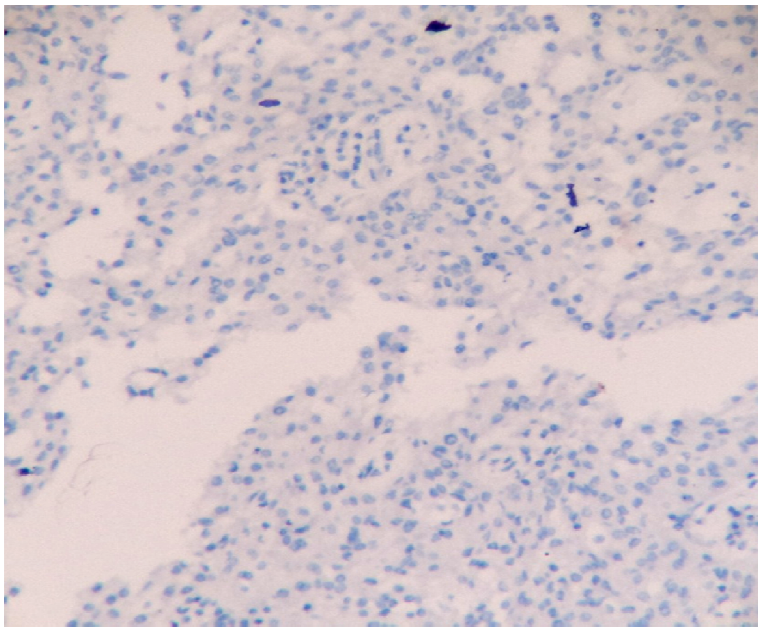

Figure 45.CK-20 expression –negative in Mucoepidermoid carcinoma(10x).

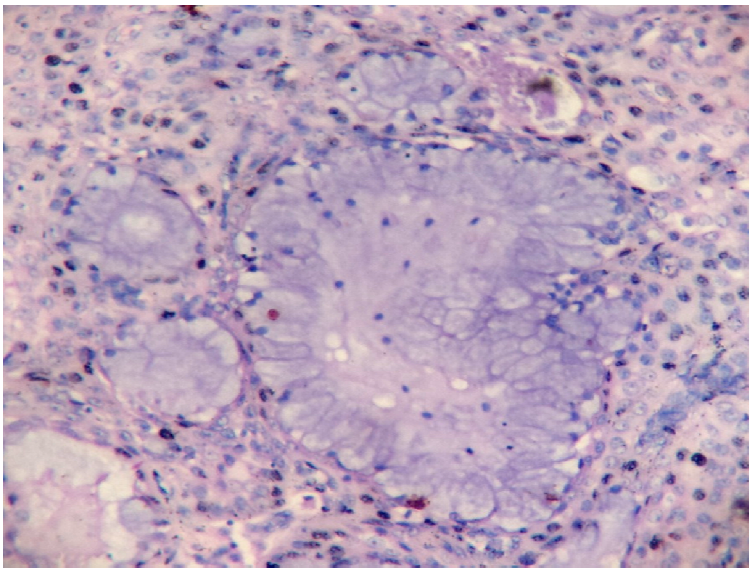

Figure.46 Ki-67 index<10% in Intermediate grade Mucoepidermoid carcinoma (10x).

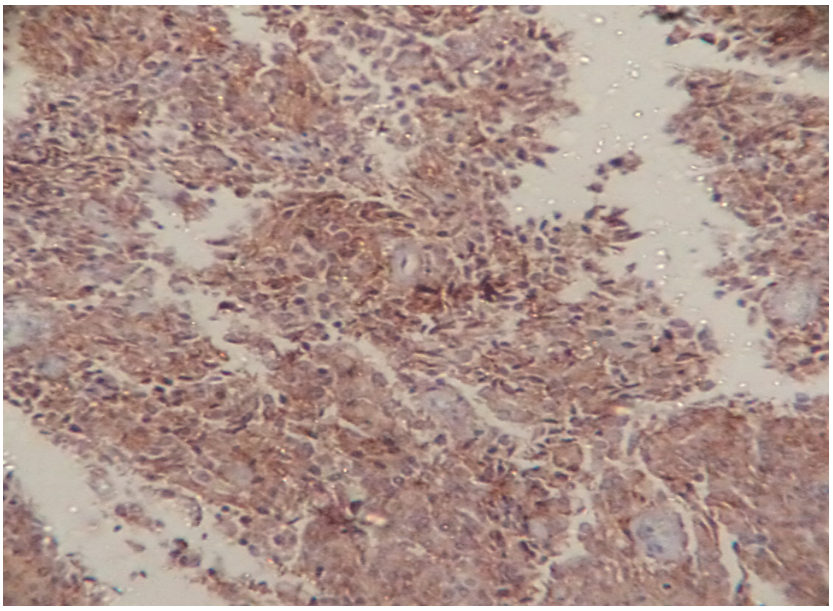

Figure 47.Her2/neu in Mucoepidermoid carcinoma shows diffuse positivity [2+].(40x).

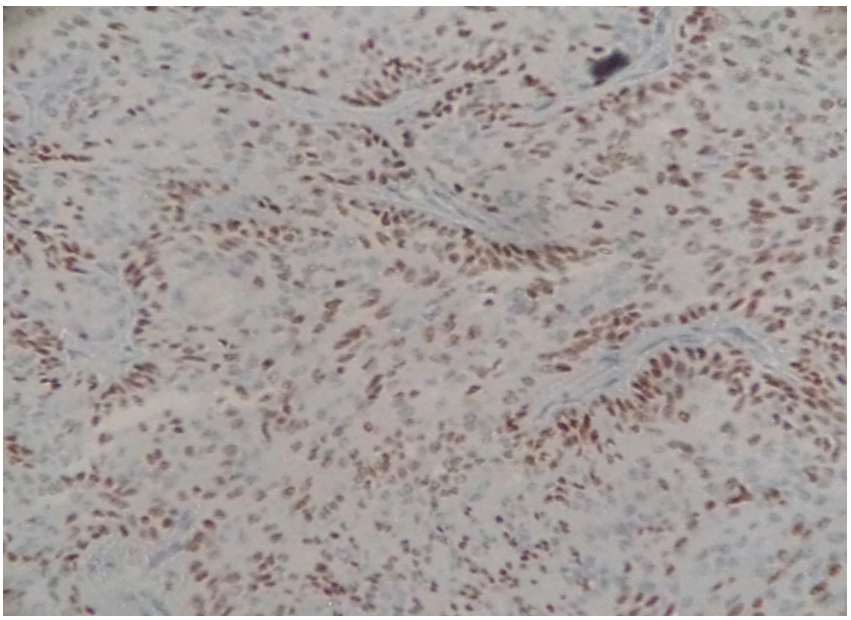

Figure 48. P 63 in Myoepithelioma-myoepithelial cells have taken p63 (10x).

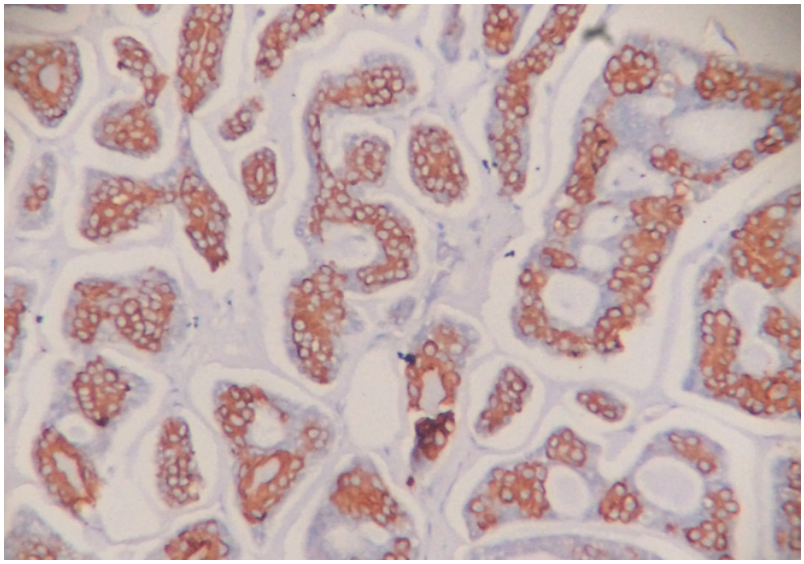

Figure 49. Cytokeratin-7 expression in Adenoid cystic carcinoma. Luminal cells are positive(10x).

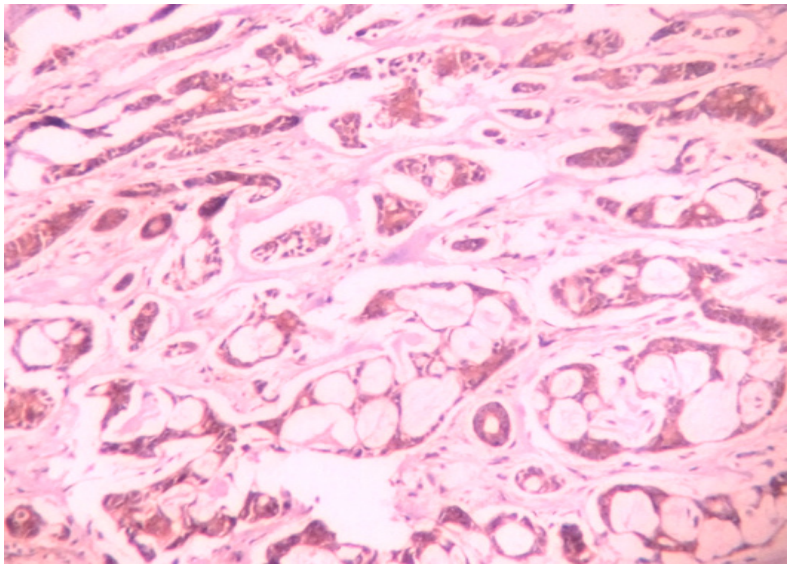

Figure 50. CD-117 expression in Adenoid cystic carcinoma. Luminal cells are positive for CD-117(10x).

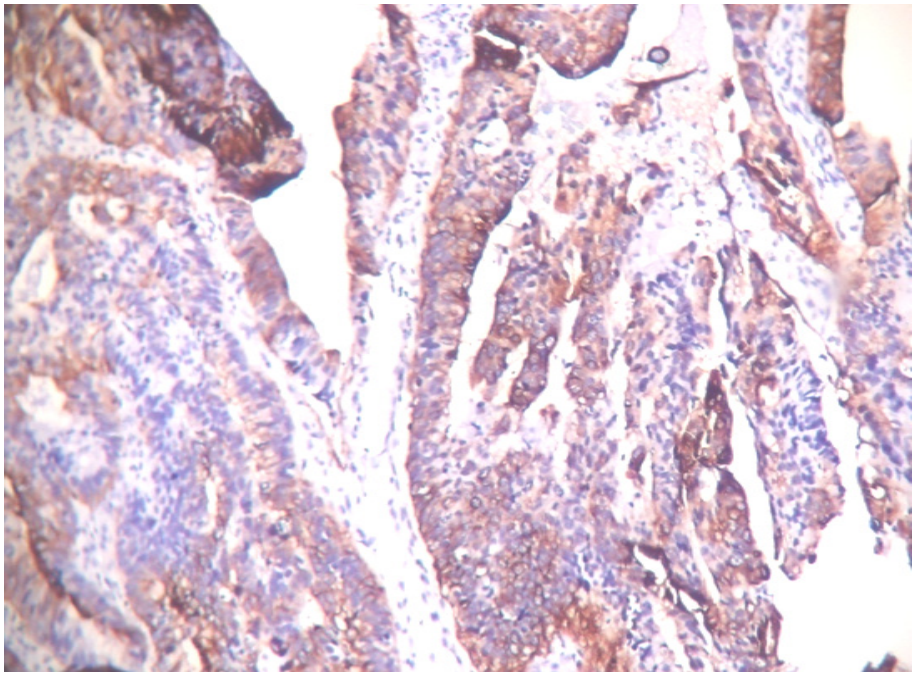

Figure 51. Cytokeratin - 7 positive in Basal cell adenocarcinoma.(10x).

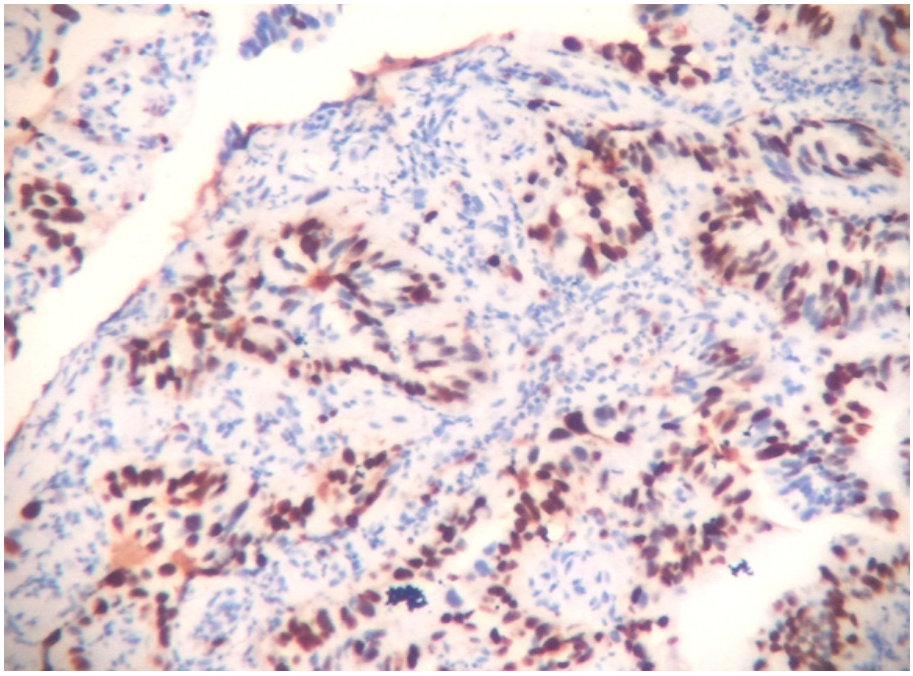

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Figure 52. Ki -67 index in Basal cell adenocarcinoma >50% (10x).

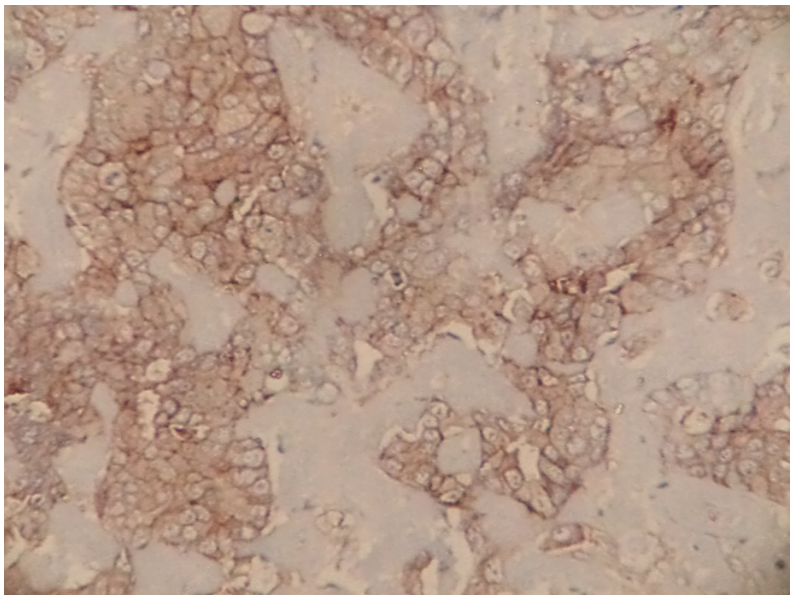

Figure 53. Her2 /neu expression in Salivary duct carcinoma shows 2+ positive.(10x)

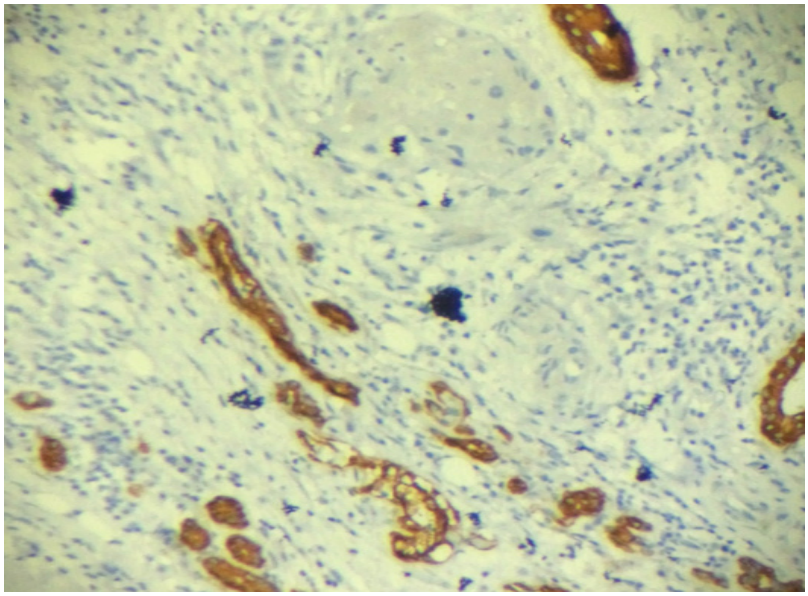

Figure 54.CK-7 expression negative in Primary squamous cell carcinoma.(10x).

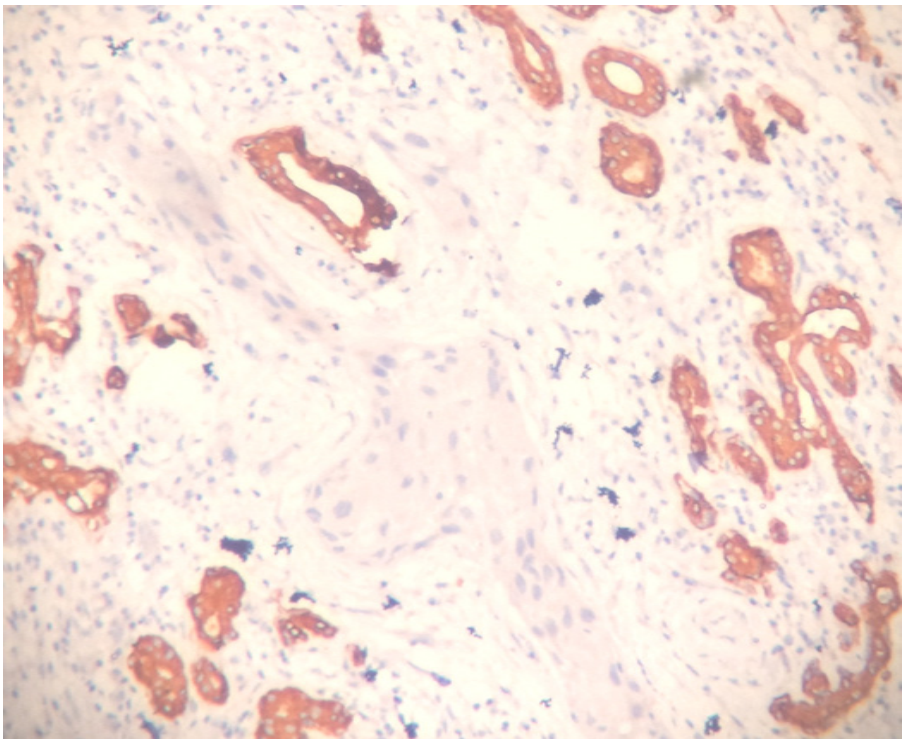

Figure 55. CK-20 expression negative in Squamous cell carcinoma.(10x).

DISCUSSION

The present study was conducted in the Department of pathology, Thanjavur medical college for a period of three years. A total of 92 cases were studied with respect to incidence,age, sex,incidence, gross and microscopic features.

I NCIDENCE PER YEAR OF SALIVARY GLAND TUMORS IN DIFFERENT SERIES

TABLE19:

SERIES	TOTAL NO OF TUMORS	PERIOD OF STUDY	NO OF CASES PER YEAR
Vuhahula et al[108]2004	268	10	26.8
Ito et al[109]2005	496	29	17
ThomasKM [110]et al	190	9	21
Otoh et al [111]	202	10	20.2
Present study	64	3	21.33

In the present study incidence of salivary gland tumor per year was 21.33% which correlates with Thomas et al and Otoh et al. Vuhahula et al[108] has got a higher incidence than ours.Ito et al[109] observed a very low incidence[chart-14].

Table:20

FREQUENCY OF BENIGN AND MALIGNANT TUMORS

SERIES	TOTAL CASES	BENIGN	MALIGNANT
Ito et al [109]	335	67.5%	32.5%
Vuhahula EA et al[108]	125	53.4%	46.4%
Jones et al[113]	741	64.9%	35.1%
Otoh et al [111]	202	63.00%	37.00%
Gupta et al[114]	113	69.90%	30.10%
Our study	64	64.06%	35.94%

The frequency of occurrence of benign tumors were more common when compared to malignant tumors which was observed in present study as well as in the above series in the present study the incidence of benign tumor was 64.06% and 35.94% for malignant tumors [chart-15].

AGE DISTRIBUTION OF TUMORS IN DIFFERENT SERIES

TABLE 21:

SERIES	BENIGN	MALIGNANT
Thomas et al[110]	39	46
Krishnaraj subha raj[115]	43	56
Ahmed et al[75]	32.7	43.1
Rewusuvan et al [117]	72	49
Satko et al[118]	47.41	52.59
Felipe[112]	46.3	54
Present study	43.39	49.95

In most of the studies benign tumors occur at a younger age group than compared to malignant tumors. The results in the above series are correlated with other Indian studies .

The mean age of benign tumor was 43.39% and for malignant tumor was 49.95% the present study correlates with krishnaraj subharaj,[115]Felipe PF,[112] Rewusuvan et al[117] and satko et al[118][chart-16]

TABLE 22: SEX DISTRIBUTION OF TUMORS IN DIFFERENT SERIES

SERIES	TOTAL	BENIGN(M:F)	MALIGNANT(M:F)
Mohb Ayubet al[119]	1:1.4	1.9:1	1:3.3
Budharaj et al[120]	1.1:2.0	1.0:1.0	1.0:2.0
Gupta et al[114]	1.0:1.7	1.0:1.6	1.0:1.2
Rewusuvan et al[117]	1.0:1.3	1:1.2	1:1.5
Present study	1:2.56	1:3.1	1:1.8

In our study there was slight female preponderance in both benign and malignant tumors, similar to other studies.However Mohb Ayub et al[119] have observed a male preponderance.

TABLE 23: SITE DISTRIBUTION OF SALIVARY GLAND TUMORS IN VARIOUS STUDIES

SERIES	PAROTID	SUBMANDIBULAR	MINOR SALIVARY GLAND
Arruda Morias et al[122]	72.6%	15.2%	12.2%
Ahmed et al[116]	70%	18%	12%
Davis et al[123]	75%	13.7%	11.3%
Gupta et al[114]	67.80%	16.10%	16.15%
Rewusuvan et al[117]	79%	18%	2%
Chaterjee & Panda[124]	70%	18%	12%
Felepi PF et al[112]	42.3%	6.8%	26.9%
Our study	76.56%	4.68%	18.76%

In the present study, parotid was the commonest site with the incidence of 76.56%, followed by minor salivary gland with an incidence of 18.76%, submandibular gland with an incidence of 4.68%. Similar results were observed by Rewusuvan et al[117]and Davis et al[123] [chart17].

TABLE 24:INCIDENCE OF PLEOMORPHIC ADENOMA IN VARIOUS STUDIES

SERIES	TOTAL NO OF TUMORS	PLEOMORPHIC ADENOMA	% OF TOTAL TUMORS	% OF BENIGN TUMORS.
Mohb Ayub et al[119]	204	127	62.25%	74.5%
Rewusuvan et al[117]	198	84	64.12%	72.78%
Vuhahula et al[108]	268	107	39.93%	73.8%
Skolnik et al[125]	435	221	50.80%	73.20%
Ito et al[109]	496	397	54.20%	80.30%
Jones et al[113]	741	329	44.4%	68.4%
FelipePF et al[112]	493	73	63.60%	85.0%
Our study	64	30	46.87%	73.17%

In the present study Pleomorphic adenoma was the most common tumor accounting for 73.17% of benign tumors and 46.87% of all tumors.This is similar to the results of other studies[chart-18].

TABLE25 : SEX AND AGE DISTRIBUTION OF PLEOMORPHIC ADENOMA

SERIES	TOTAL NO OF TUMORS	PA	PEAK AGE	M:F
Mohb Ayub et al[119]	204	127	31-50	1:2.3
Ahmed et al[116]	100	86	30-50	1.1:1.0
Sharkey FE[126].	338	203	20-40	1:1.8
Rewusuvan et al[117]	198	84	14-79	1:2.1
Thomas et al[110]	190	113	30-50	1:1.1
Felipe PF et al[112]	493	493	31-50	1:1.55
Our study	64	30	30-50	1:4

The age incidence in present study correlates with studies of Mohb Ayub et al[119]and Ahmed et al.[116] The peak age incidence was 4th decade in the present study. However Rewusuvan et al[117]observed a higher age incidence. Female preponderance was seen in cases of Pleomorphic adenoma in the present study similar to other studies.

TABLE 26: LOCATION OF PLEOMORPHIC ADENOMA IN VARIOUS SITES

SERIES	PAROTID	SUBMANDIBULAR	MINOR SALIVARY GLAND
Buddhraja et al[120]	82.80%	13.80%	3.40%
Otoh et al[111]	80.00%	11.10%	18.00%
Renehan et al[127].	93.00%	3.4%	3.6%
Rewsuvan et al[117]	79.21%	28.5%	2.38%
Our study	90%	-	10%

Parotid is the most common site for Pleomorphic adenoma followed by minor salivary glands. This was in accordance with other studies. No case was recorded in submandibular gland in the present study[chart-19].

PATHOLOGY:

GROSS: PA ranges in size from 1 to 6cms with solid, glistening, grey white areas [fig-1] and with focal myxoid areas.[fig-5] which correlates with other studies .

MICROSCOPY: Shows features composed of myoepithelial, epithelial, myxoid and chondroid areas.[figure4,5]The epithelial components are composed tubules, ribbons,, small cysts and solid sheets. The cells were columnar to cuboidal.

The duct lumen contains eosinophilic material which are PAS positive [figure-10]. The myoepithelial cells were spindle, stellate, cuboidal and plasmacytoid arranged in sheets trabeculae. Stellate and spindle cells were suspended in abundant myxoid stroma.

Six of the cases showed chondroid differentiation[figure-4],two cases showed lipomatous areas [figure-7],three casess showed squamous metaplasia [figure-3] and one case showed sebaceous differentiation[fig-6].

Everson J W noted squamous metaplasia in pleomorphic areas. PAS was positive in mucinous areas ,alcian blue stain was positive for chondromyxoid areas[figure8].

TABLE 27:INCIDENCE OF BASAL CELL ADENOMA IN VARIOUS STUDIES

SERIES	TOTAL NO OF TUMORS	BASAL CELL ADENOMA	% OF BENIGN TUMORS	% OF TOTAL TUMORS
Krishnaraj subhashraj[115]	684	11	2.6%	1.6%
Nagakar et al[128]	36	1	3.70%	2.77%
Our study	92	6	14.36%	9.37%

In this study, six cases of Basal cell adenoma was found which accounts for 14.36% of benign tumors and 9.37% of of all salivary gland tumors. Basal cell adenoma is the second most common benign tumor in our study. Incidence of Basal cell adenoma in our study was higher than studies of Nagarkar et al.[128]

Our study shows a female preponderance with four cases reported in parotid and two cases in minor salivary gland[chart-20].

PATHOLOGY :

GROSS: Excision biopsy shows a well circumscribed tumor with solid homogenous grey white,firm nodule [figure-11].

MICROSCOPY: Tumors are composed of small ,round, uniform basaloid cells with large centrally placed nuclei, scant basophilic cytoplasm arranged in cords, trabeculae and sheets[figure-13] separated by a fibrovascular stroma. Similar findings were recorded by Evans et al[134].

TABLE 28:INCIDENCE OF WARTHINS TUMOR IN VARIOUS STUDIES

SERIES	TOTAL NO OF TUMORS	NO OF WARTHINS CASES	% OF BENIGN TUMORS	% OF ALL TUMORS
Khazanchi et al[129]	88	06	10.70%	6.80%
Nagarkar et al[128]	36	02	7.4%	5.55%
FelipePF[112]	493	4	9.76%	7.3%
Present study	64	4	9.76%	6.25%

Warthin's tumor was the third most common benign tumor in our study accounting for 6.25% of all salivary gland neoplasms and 9.76 % of benign tumors.

This correlates with the results of Felipe,[112] khazanchi et al,[129] while Nagarkar et al[128] noted a low incidence [chart-21].

TABLE 29:AGE AND SEX DISTRIBUTION OF WARTHIN'S TUMOR

SERIES	TOTAL NO TUMORS	WARTHIN'S TUMOR	PEAK AGE	M:F
Sharkey[126] et al	338	61	40-60	2.8:1
Skolnik [125]et al	435	46	60-70	4.7:1
Rewuswan[117] et al	198	38	40-60	2.1:1
Felipe [112]et al 2012.	493	36	40-70	3:1
Present study	64	4	40-60	4:1

The peak age distribution of warthin's tumor in present study was between 40-60 years. Our study correlates with sharkey et al,[126] rewuswan et al.[117] Male predominance is noted in our study similar to Sharkey et al,[126] Skolniket et al, [125] Felipe [112][2012].

GROSS: Warthins tumor shows grey white areas with slit like spaces with papillary projection[figure 14].

Warthin,s tumor is characterized by double layer of cells, the outer layer of columnar cells and inner layer of oncocytic cuboidal cells arranged in papillary and glandular pattern. Stroma showed abundant lymphoid tissue with formation of lymphoid follicles[figure-15].

TABLE 30:LOCATION OF WARTHIN'S TUMOR

SERIES	NO OF WARTHINS TUMOR	PAROTID	SUBMANDI BULAR	MINOR SAL
Budhraja et al[120]	2	2	-	-
Skolnik et al[125]	46	46	-	-
Mohb Ayub et 9al[11]	12	12	-	-
Felipe[112]	30	29	1(3.34%)	-
Present study	04	04	-	-

Our study showed four cases of warthin's, all occurred in parotid gland, this is similar to results from other studies done by Budhraja et al,[120] Skolnik et al[125] and Felipe[112] [2012][chart-22].

MYOEPITHELIOMA:

One case of myoepithelioma was seen in 31 year old female accounting for 1.5% of all salivary gland tumors and 2.44% of all benign tumors. This tumor was seen in minor salivary gland. This study correlates with Jones et al [113].

Microscopy: The tumor cells shows spindle cells[figure 16] with central vesicular nuclei and eosinophilic cytoplasm with occasional duct formation similar to the observation of Dardick I et al.[130]

TABLE 31: INCIDENCE OF MUCOEPIDERMOID CARCINOMA(MEC) IN VARIOUS STUDIES

SERIES	TOTAL NO TUMORS	MEC	% OF ALL TUMORS	% OF MALIGNANT TUMORS
Vahahula et al [108]	268	25	21.6%	46.6%
Vargas et al[131]	124	13	10.48%	52.0%
Panda et al[124]	315	26	8.26%	39%
Felipe [112]	493	39	7.9%	31.4%
Our study	64	12	7.9%	52.17%

In our study 12 cases of mucoepidermoid carcinoma were seen comprising 7.9% of all salivary gland tumors and 52.17% of all malignant tumors. Vargas et al[131] in their study of 124 tumors, Mucoepidermoid carcinoma constitutes 10.48% of all tumors. Panda et al,[124] studied 315 cases of salivary gland of these, Mucoepidermoid constitutes 8.26%.[chart-23].

TABLE 32: AGE &SEX INCIDENCE OF MEC IN VARIOUS STUDIES

SERIES	TOTAL NO OF TUMORS	TOTAL NO CASES	PEAK AGE	M:F
Joneset al al[113]	741	85	51-60	1:1.6
Vargas et al[131]	124	85	51-60	1:1.6
Felipe[112]	493	39	31-70	1:1.33
Present study	64	12	40-60	1:3

Present study shows a slight female predominance . Similar observation was made by Jones et al,[113] Felipe[112][2012]. The peak age incidence of Mucoepidermoid carcinoma is seen in the age group of 40-60 years. The youngest age in our study is a 7 year old female child.

TABLE 33: LOCATION OF MEC IN VARIOUS STUDIES

SERIES	PAROTID	SUBMANDIBULAR	MINOR SALIVARY GLAND
Nagarkar et al [128]	80%	-	-
Agarwal et al [132]	78.6%	21.4%	-
Sharkey et al[126]	80.00%	4.00%	16%
Ochicha et al[133]	49%	26%	24%
Present study	100%	-	-

Parotid gland is the most common site for Mucoepidermoid carcinoma in our study. Observations are similar to Nagarkar et al.[128] In our study all the cases were seen in parotid and none from minor salivary gland similar to Nagarkar et al,[128]Agarwal et al.[132]

TABLE 34:HISTOLOGICAL GRADING OF MEC

SERIES	NO OF MEC	LOW	INTERMEDIATE	HIGH
Renehan et al[127]	38	87.4%	-	2.6%
Goode et al[134]	234	76.06%	7.69%	13.24%
Present study	12	83.34%	8.33%	8.33%

The present study showed a preponderance of low grade mucoepidermoid carcinoma[83.34%], similar to results of renehan et al,[127]Goode et al[134][chart-24].

GROSS: Grey white to pink areas with,cystic spaces in low grade cases [fig-17] and predominantly solid areas in high grade cases[fig-18].

MICROSCOPY: In our study, tumors showed three types of cells such as mucous cells, epidermoid cells and intermediate cells[figure20-23]. Mucous cells are PAS positive[fig-19]. Lymph node metastasis was seen in a case of high grade Mucoepidermoid carcinoma.

TABLE35:INCIDENCE OF ADENOID CYSTIC CARCINOMA IN VARIOUS STUDIES

SERIES	TOTAL NO OF TUMORS	ADENOID CYSTIC CARCINOMA	% OF TOTAL TUMORS	% OF MALIGNANT TUMORS
Rewusuwan et al [117]	198	18	10	24.74%
Nagarkaret al[128]	36	2	5.5%	25%
Vargas[131] et al	124	5	4.03%	20.0%
Felipe[112] [2012]	493	12	7.4%	16.4%
Present study	64	5	7.81%	21.73%

In the present study Adenoid cystic carcinoma constituted 7.81% of all tumors of salivary gland tumors and 21.73% of malignant salivary gland tumors. Similar results were obtained in studies of Felipe[112][2012] .

However Rewusuvan et al [117] observed 18 cases out of 198 salivary gland tumors which showed slight higher incidence than other studies.

TABLE 36: AGE AND SEX INCIDENCE OF ADENOID CYSTIC CARCINOMA

SERIES	TOTAL NO OF CASES	ADENOID CYSTIC CARCINOMA	PEAK AGE	M:F RATIO
Rewusuvanet al [117]	198	18	40-50	1:1.5
Agarwal et al[132]	147	11	35-64	1.2:1.0
Sharkey FE[126]	338	07	41-60	1.0:1.3
Filipe[112]	493	12	31-70	3:2
Present study	64	5	41-60	1:0.66

Present study showed age range from 41-60 years with mean age of 40.87 years which corresponds to Sharkey FE et al[126] and Rewusuvan et al[117] with a mean age of 46.38 years . Our study showed a slight male preponderance compared to other studies.

TABLE 37: SITE DISTRIBUTION OF ADENOID CYSTIC CARCINOMA

SERIES	PAROTID	SUBMANDIBULAR	MINOR SALIVARY GLANDS
Vihuhala et al[108]	16.3%	37.0%	38.3%
Pour et al[135]	46.70%	0.67%	46.60%
Present study	1.56%	3.125%	3.125%

In the present study only one case occurred in parotid which accounts for 1.56% and two cases each occurring in submandibular and minor salivary gland accounting for 3.125% each. Other studies showed a preponderance of minor salivary gland for Adenoid cystic carcinoma.

Microscopy: Showed predominantly cribriform and tubular pattern with basaloid cells with angulated hyperchromatic nuclei with scant cytoplasm separated by a hyaline stroma [figure 25].Similar findings were obtained by Auclair Ellis and Gnepp. A variable combination of all three patterns can occur in same tumor. One case shows extensive hyalinization.

SALIVARY DUCT CARCINOMA:

In our study two cases of salivary duct carcinoma was reported one in submandibular gland, other in minor salivary gland in the age group of 50-70 years. The incidence was 8.70% of malignant tumors and 3.13% of all salivary gland tumors.

MICROSCOPY: Showed intraductal component with cribriform, solid, papillary pattern with central comedonecrosis as stated by Nagao et al. [82] Our case showed ductal components with central comedo necrosis.[fig- 27]

Meyers and Ferris et al [2007] reported a incidence of 9% in his studies.

CARCINOMA EX PLEOMORPHIC ADENOMA:

Felipe[112] [2012] in their series of 493 cases observed an incidence of 1.6%[malignant tumors] of ca ex pleomorphic adenoma.

In another study by Mag[135] et al [2010] an incidence of 28.57% was reported .

In our study 2 cases of Carcinoma ex pleomorphic adenoma were reported with an incidence of 8.70% of malignant tumors and 3.13% of all salivary gland tumours.

Microscopy: Showed an infiltrative growth composed of pleomorphic cells with hyperchromatic nuclei and moderate amount of cytoplasm arranged singly and in groups amidst myxoid stroma.

BASAL CELL ADENOCARCINOMA

In our study one case of basal cell adenocarcinoma of 70 years of age was reported which accounts for 4.34% of malignant tumors and 1.56% of all salivary gland tumors.

Histopathological examination reveals basaloid cells[fig-26] arranged in solid and tubular arrangements with infiltrating pattern as stated by barnes et al.[136] This tumor arise primarly from parotid[80%]of older individual, most cases arise de novo[77%] but some cases arise from pre existing basal cell adenoma[23%].

PRIMARY SQUAMOUS CELL CARCINOMA :

Primary squamous cell carcinoma can occur in salivary gland. It accounts for 1.6% of primary epithelial salivary gland tumors with a mean age of 60.5 years.[41]

In the present study a single case was reported with an incidence of 4.34% of malignant tumor and 1.56% of all salivary gland tumor. This case occur in submandibular region [figure-28].

Rasp G et al [141]reported a primary squamous cell carcinoma in a child .In the present study we reported a case of 70 years female.

NON NEOPLASTIC LESIONS

MUCOUS RETENTION CYST:

In our study we reported 14 cases of mucous retention cyst which accounts for 50% of all non neoplastic lesions. All the cases occur in minor salivary gland with a slight female predominance.

MICROSCOPY: Showed cyst lined by cuboidal to columnar epithelium with lumen filled with eosinophilic material[fig-29], inflammatory material composed of neutrophils, lymphocytes and histiocytes similar to the findings of sajeevan TP et al[2] .

CHRONIC SIALADENITIS:

Among 28 non neoplastic lesions, chronic sialadenitis[fig-30] accounts for 8 cases which accounts for 28.57% of all non neoplastic lesions. Of the 8 cases five cases occur in submandibular gland (17.85%) and 3 cases occur in parotid(10.72%) with a male predominance .

Seifert et al[142] stated the etiological classification of sialadenits

CHRONIC SCLEROSING SIALADENITIS:[KUTTNER TUMOR]

In our study we reported a single case in submandibular region which constitutes 3.57% of non neoplastic cases.

Kuttner tumor occurs exclusively in the submandibular region as stated by Sadayuki. K B et al[138]

Wah cheuk and chan [139] stated that kuttner tumor is characterized histologically by preservation of lobular architecture, lymphoid follicle formation, thickening of inter lobar septa by sclerosed tissue,variable loss of acini and preservation of acini with periductal fibrosis.

IMMUNOHISTOCHEMISTRY IN SALIVARY GLAND TUMORS:

p63 IN PLEOMORPHIC ADENOMA AND MUCOEPIDERMOID CARCINOMA:

In present study, four cases of pleomorphic adenoma were selected for myoepithelial marker p63 [figure-35,36]. All the cases the abluminal cells have taken p63, which implies the origin of pleomorphic adenoma from intercalated duct component.

In mucoepidermoid carcinoma IHC was done with p63. All the cases, intermediate epidermoid cells have taken p63, [fig 44] but not the myoepithelial cells. This confirms the origin of MEC from excretory or striated duct component of salivary gland

Similar results were observed by Batsakis et al[143] ,Loyala and souse et al[144][1998] Marucci and Foschini[145] [2002] et al.[145]and Nago et al [65].

MARKERS IN BENIGN TUMORS

CK-7 was taken by the luminal cells[fig-37] of Pleomorphic adenoma as reported by Naga et al[65].

Ki-67 index in PA was 1% in present study[fig-38] which correlates with studies done by Anna kananceva et al[140]

In Basal cell adenoma the luminal cells are positive for CK-7 [figure-39] and surrounding myoeppithelial cells are positive for P63[fig-40] which correlates with the studies of Edwards PC et al.[146] Ki-67 labelling index was <3% which correlates with Everson and Nago et al[10].

Warthin's tumor luminal cells are positive for CK-7[fig-41] and basaloid cells are positive for p63[fig-42] as stated by Peigu et al studies.[19]

In present study a single case of myoepithelioma was stained with P63[fig-48],which has taken the nuclear staining as stated by Dardick et al[147] [1989].

MARKERS IN MALIGNANT SALIVARY GLAND TUMORS

In Mucoepidermoid carcinoma CK-7 has taken up by the mucocytes and intermediate cells [fig- 43] as stated by Maruya et al.[70] In present study, MEC was positive for CK-7 [fig-43] and negative for CK-20[fig 45] stated by NikitakisN G et al[137].

Ki 67 labelling index in low grade MEC was found to be negative, whereas in intermediate MEC Ki-67 labelling index was found to be 10%.[fig-46]

In MEC when the Ki-67 Index is < 5% there was no recurrence, when Ki-67 index >10% associated with poor out come[65].

In present study HER2/neu expression was done in one case of MEC-intermediate grade and it was found to diffuse and cytoplasmic membrane positivity with grade 2 positive[fig -47].

Press MF[148] et al stated that 40% cases of MEC are positive for HER2/neu and these cases are associated with poor prognosis.

In Adenoid cystic carcinoma , the luminal cells was found to be positive for CK-7[fig -49] and the basal cells were positive for p63 as stated by Chen JC et al[49][1988] .

Luminal cells of Adenoid cystic carcinoma are intensively positive for CD-117[fig-50] as stated by Holst VA[150] et al.

Amoueian [151]et al stated that Ki-67 expression in Adenoid cystic carcinoma was found to be in the range of 0-85%.

In present study one case of basal cell adenocarcinoma was found to be CK-7 positive [fig-51] and Ki-67 index of >50%[figure-52]as stated by Nago et al[152].

In present study 2 cases of salivary duct carcinoma shows diffuse and strong membraneous staining for HER2/neu[fig-53] as stated by Jaehne [153] et al. Similar results were observed by Etges1 et al[154] [2003].

In present study a single case of primary squamous cell carcinoma shows CK-7 and CK-20 negative[fig-54,55] as stated by Nikitakis NG et al[137] 2004 .

CONCLUSION

Out of 13,916 general biopsies received in Thanjavur medical college during September 2012- August 2014 , 92 cases were salivary gland lesions accounting for an incidence of 0.6%.

1. Peak incidence of benign salivary gland lesion was in the age group of 30-50 years and in malignant lesions it was in the age group of 30-70 years.
2. The mean age for benign tumors was 43.39 years and in malignancy it was 49.95 years.
3. Salivary gland lesions shows a female preponderance with male to female ratio of 1 : 1.7.
4. Among the 64 cases of salivary gland tumors majority were found in the parotid accounting for 76.56% followed by minor salivary gland 18.76% and submadibular gland 4.86%.
5. Inflammatory lesions were more common in minor salivary gland followed by submandibular gland. Benign tumors were more common in parotid whereas in minor salivary glands, malignant tumors were more common.
6. Most common benign salivary gland tumor in present study is Pleomorphic adenoma with an incidence of 73.17% followed by Basal cell adenoma which accounts for 14.63% .
7. Most common malignant salivary gland tumor was Mucoepidermoid carcinoma accounting for 52.17% of malignant tumors followed by Adenoid cystic carcinoma.

8. Expression of p63 in Pleomorphic adenoma had confirmed the role of myoepithelial cells in the histiogenesis of this tumor and lack or minimal expression of p63 in Mucoepidermoid indicates minimal myoepithelial cell differentiation .
9. Cytokeratin -7 is expressed in the luminal cells of Pleomorphic adenoma, Basal cell adenoma, Warthin's tumor, Adenoid cystic carcinoma and Basal cell adenocarcinoma.
10. Ki-67 is the most frequently used prognostic markers in malignant salivary gland tumors. HER2/neu is also used as a prognostic marker in Salivary duct carcinoma.
11. Histopathology is the gold standard for the diagnosis of salivary gland tumors. Immunohistochemistry plays a limited, even though important role in the diagnosis of salivary gland tumors when the diagnosis is uncertain.

LIST OF ABBEREVATIONS USED

AdCC-Adenoid cystic carcinoma

AR- Androgen receptor

BCA-Basal cell adenoma

Ca- carcinoma

CEA- carcinoembryonic antigen.

CK-cytokeratin

C ex PA-carcinoma ex Pleomorphic Adenoma

E-Eosin

ER-Estrogen receptor

EMA-Epithelial membrane antigen

FNAC-Fine needle aspiration cytology

GFAP-Glial fibrillary acid protein

H- Hematoxylin

IHC-Immunohistochemistry

LESA;-Lymphoepithelial sialadenitis

MEC-Mucoepidermoid carcinoma

MUC-Mucin

PA-Pleomorphic adenoma

PAS-Periodic acid Schiff

PLGA-Polymorphous low grade adenocarcinoma.

PR- Progesterone receptor.

SCC- Squamous cell carcinoma.

SDC- Salivary duct carcinoma

SMA-Smooth muscle Actin

WHO-World health organization

MASTER CHART (Annexure - I)

SNO	HPE NO	AGE	SEX	SITE	HPE DIAGONOSIS	NEOPLASM/ NON NEOPLASM	BENIGN/MALIGNANT	IHC MARKER
1	2191/11	30	F	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Malignant	
2	2385/11	27	M	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
3	2543/11	43	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
4	2546/11	64	M	Parotid	warthin's tumor	Neoplasm	Benign	
5	3o11/11	30	F	Hard palate growth	Basal cell Adenoma	Neoplasm	Benign	
6	3051/11	38	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
7	3912/11	55	F	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Malignant	
8	3942/11	58	F	Oropharynx	Adenoid Cystic Carcinoma	Neoplasm	Malignant	
9	4476/11	37	M	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Malignant	
10	4313/11	39	F	Parotid	Pleomorphic Adenoma	Neoplasm	benign	
11	4514/11	56	M	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
12	4568/11	63	F	Parotid	Carcinoma ex pleomorphic adenoma	Neoplasm	Malignant	
13	462/12	40	F	Maxilla	Adenoid Cystic Carcinoma	Neoplasm	Malignant	CD-117
14	491/12	25	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
15	548/12	70	M	Soft palate	Salivary duct Carcinoma	Neoplasm	Malignant	
16	589/12	70	F	Submandibular	Primary squamous cell carcinoma.	Neoplasm	Malignant	CK-7,CK-20
17	879/12	45	F	Parotid	Pleomorphic Adenoma	neoplasm	Benign	

SNO	HPE NO	AGE	SEX	SITE	HPE DIAGONOSIS	NEOPLASM/ NON NEOPLASM	BENIGN/MALIG NANT	IHC MARKER
18	914/12	34	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
19	1246/12	32	M	Parotid	Adenoid Cystic Carcinoma	Neoplasm	Malignant	
20	1260/12	58	M	Submandibular	Adenoid Cystic Carcinoma	Neoplasm	Malignant	
21	1374/12	65	F	Soft palate	Pleomorphic Adenoma	Neoplasm	Benign	
22	1414/12	51	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
23	1458/12	40	M	Submandibular	Chronic sialadenitis	Non Neoplasm		
24	1547/12	62	M	Submandibular	Chronic sialadenitis	Non Neoplastic		
25	1592/12	54	F	Parotid	Mucoepidermoid Carcioma	Neoplasm	Malignant	
26	1643/12	45	M	Submandibular	Chronic sialadenitis	Non Neoplasm		
27	1837/12	32	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
28	1865/12	33	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
29	2780/12	17	M	Lip	Mucous Retension Cyst	Non Neoplasm		
30	2836/12	11	M	Parotid	Parotid Pneumatocele	Non Neoplasm		
31	2989/12	45	F	Parotid	Basal Cell Adenoma	Neoplasm	Benign	
32	3052/12	50	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
33	3222/12	24	M	Parotid	Kimura's Disease	Non Neoplasm		
34	3225/12	50	M	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	

SNO	HPE NO	AGE	SEX	SITE	HPE DIAGONOSIS	NEOPLASM/ NON NEOPLASM	BENIGN/MALIG NANT	IHC MARKER
35	3301/12	26	F	Floor of Mouth	Mucous Retension Cyst	Non Neoplasm		
36	3367/12	41	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
37	3969/12	38	M	Submandibular	Chronic sialadenitis	Non Neoplasm		
38	4657/12	33	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
39	4905/12	65	M	Parotid	warthin's tumor	Neoplasm	Benign	CK-7,P63
40	5003/12	45	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
41	136/13	37	F	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Malignant	
42	190/13	51	F	Submandibular	Salivary duct Carcinoma	Neoplasm	Malignant	Her2/neu
43	286/13	70	M	Submandibular	Kuttners Tumor	Non Neoplastic		
44	337/13	46	M	Lip	Mucous Retension Cyst	Non Neoplasm		
45	731/13	38	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
46	824/13	50	F	Parotid	Basal cell Adenoma	Neoplasm	Benign	CK-7,P63
47	970/13	24	M	Lower Lip	Mucous Retension Cyst	Non Neoplasm		
48	1205/13	41	M	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Malignant	
49	1315/13	23	F	Parotid	Monomorphic Adenoma	Neoplasm	Benign	
50	1357/13	75	F	Parotid	Mucoepidermoid Carcinoma High Grade	Neoplasm	Malignant	
51	1574/13	45	F	Parotid	Pleomorphic Adenoma-Cellular	Neoplasm	Benign	

SNO	HPE NO	AGE	SEX	SITE	HPE DIAGONOSIS	NEOPLASM/ NON NEOPLASM	BENIGN/MALIG NANT	IHC MARKER
52	1680/13	55	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
53	1752/13	48	F	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Malignant	
54	1809/13	55	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
55	1817/13	25	F	Parotid	Simple Serous Cyst of Parotid	Non Neoplasm		
56	1876/13	80	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
57	1894/13	31	F	Cheek	Myoepithelioma	Neoplasm	Benign	P63
58	1910/13	42	F	Parotid	Chronic sialadenitis	Non neoplastic		
59	1952/13	44	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
60	2142/13	23	M	Parotid	Pleomorphic Adenoma-Recurrent	Neoplasm	Benign	P63
61	2440/13	60	F	Parotid	Lymphoepithelial Cyst	Non Neoplasm		
62	2463/13	10	F	Lip	Mucous Retension Cyst	Non Neoplasm		
63	2562/13	35	F	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Malignant	P63.
64	2677/13	65	F	Maxilla	Monomorphic Adenoma	Neoplasm	Benign	P63,Ki-67
65	3186/13	18	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	
66	3376/13	13	M	Lip	Mucous Retension Cyst	Non Neoplasm		
67	3400/13	80	F	Cheek	Carcinoma ex pleomorphic adenoma	Neoplasm	Malignant	Her2/neu
68	3572/13	23	M	Parotid	Parotid cyst	Non Neoplasm		

SNO	HPE NO	AGE	SEX	SITE	HPE DIAGONOSIS	NEOPLASM/ NON NEOPLASM	BENIGN/MALIG NANT	IHC MARKER
69	3607/13	24	F	Oropharynx	Mucous Retension Cyst	NonNeoplasm		
70	3683/13	67	M	Parotid	Warthin's tumor	Neoplasm	Benign	
71	3809/13	10	F	Lower Lip	Pleomorphic adenoma	Neoplasm	Benign	
72	3859/13	15	F	Lip	Mucous Retension Cyst	NonNeoplasm		
73	3900/13	67	M	Submandibular	Adenoid Cystic Carcinoma	Neoplasm	malignant	ck-7,CD-117
74	3980/13	70	M	Nasal cavity	Pleomorphic adeoma	Neoplasm	Benign	
75	744/14	15	F	Lower Lip	Mucous Retension Cyst	Non Neoplasm		
76	830/14	48	M	Parotid	warthin's tumor	Neoplasm	Benign	
77	1232/14	26	M	Parotid	Pleomorphic adeoma-Squamous Metaplasia	Neoplasm	Benign	P63
78	1281/14	45	M	Parotid	Muco epidermoid-Intermediate	Neoplasm	Malignant	CK-7,Ki-67,her/neu
79	1376/14	25	M	Lip	Mucous Retension Cyst	Non Neoplasm		
80	1410/14	65	F	Parotid	Chronic sialadenitis	Non Neoplasm		
81	1431/14	35	F	Parotid	Pleomorphic Adenoma-Cellular	Neoplasm	Benign	P63,CK-7
82	1653/14	43	F	Oral Cavity	Mucous Retension Cyst	Non Neoplasm		
83	1729/14	45	F	Parotid	Chronic sialadenitis	Non Neoplasm		
84	1773/14	14	F	Lower Lip	Mucous Retension Cyst	Non Neoplasm		
85	1790/14	46	F	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Benign	

SNO	HPE NO	AGE	SEX	SITE	HPE DIAGONOSIS	NEOPLASM/ NON NEOPLASM	BENIGN/MALIG NANT	IHC MARKER
86	1814/14	45	F	Parotid	Pleomorphic Adenoma	Neoplasm	Benign	P63
87	1941/14	45	F	Lip	Mucous Retension Cyst	Neoplasm	Benign	
88	1999/14	70	F	Floor of Tongue	Tubular Variant of ACC\Basaliod Adeno Carcinoma	Neoplasm	Malignant	CD-117,Ki-67
89	2241/14	27	M	Parotid	Benign Parotid Cyst	Non Neoplasm		
90	2249/14	7	F	Parotid	Mucoepidermoid Carcinoma Low Grade	Neoplasm	Malignant	P63,CK-7
91	2458/14	18	M	Lip	Mucous Retension Cyst	Non neoplasm		
92	2516/14	40	F	Parotid	Basal Cell Adenoma	Neoplasm	Benign	

ANNEXURE – II

PROFORMA FOR SALIVARY GLAND TUMORS

Name : Age : Sex : IP No :

WD No :

Residence : Occupation :

Unit :

Marital status : D.O.A :

No. of children : D.O.S :

Socioeconomic status : D.O.D :

Education :

HISTORY OF PRESENTING ILLNESS :

1. Swelling
   - A. Site
   - B. Side Right / Left
   - C. Unilateral / Bilateral
2. Duration
3. Onset - Acute / chronic
4. Progression – Gradual / Rapid
5. Local changes
   - A. Pain
   - B. Edema
   - C. Cellulitis
   - D. Discharging sinus
7. Associated symptoms

A. Facial nerve involvement

Drooling of saliva

Exposure keratitis

Deviation of angle of mouth

B. Inflammatory symptoms

Fever

Malaise

Pain

Anorexia

PAST HISTORY

History of similar illness before

FAMILY HISTORY

Similar complaints of 1st degree relatives

PERSONAL HISTORY

Smoking / Alcoholism

LOCAL EXAMINATION

Swelling

Skin over the swelling

Salivary duct

Movements of jaw

Examination of facial nerve

REGIONAL LYMPH NODE STATUS

Site

Size

Number

Consistency

Mobile / fixed

Ipsilateral / Contralateral / Bilateral

SYSTEMIC EXAMINATION

CVS:

RS:

Abdomen:

CNS;

INVESTIGATIONS :

Urine examination

Routine haemogram

X – Ray

USG

CT/MRI

FNAC DIAGNOSIS

HPE DIAGNOSIS

PLAN OF TREATMENT

Simple excision

Superficial parotidectomy

Radical excision

RT/CT

Signature of the Medical Officer

ANNEXURE – III

WHO HISTOLOGICAL CLASSIFICATION OF TUMORS OF THE SALIVARY GLANDS[WHO-2005]

MALIGNANT EPITHELIAL TUMORS

Acinic cell carcinoma

Mucoepidermoid carcinoma

Adenoid cystic carcinoma

Polymorphous low grade carcinoma

Epithelial – myoepithelial carcinoma

Clear cell carcinoma – not otherwise specified

Basal cell adenocarcinoma

Sebaceous carcinoma

Sebaceous lymphadenocarcinoma

Cystadenocarcinoma

Low grade cribriform cystadenocarcinoma

Mucinous adenocarcinoma

Oncocytic carcinoma

Salivary duct carcinoma

Adenocarcinoma – not otherwise specified

Myoepithelial carcinoma

Carcinoma ex pleomorphic adenoma

Carcinosarcoma

Metastasizing pleomorphic adenoma

Squamous cell carcinoma

Small cell carcinoma

Large cell carcinoma

Lymphoepithelial carcinoma

Sialoblastoma

BENIGN EPITHELIAL TUMORS

Pleomorphic adenoma

Myoepithelioma

Basal cell adenoma

Warthin's tumor

Oncocytoma

Canalicular adenoma

Sebaceous adenoma

Lymphadenoma

- Sebaceous
- Non-sebaceous

Ductal papillomas

- Inverted ductal papilloma
- Intraductal papilloma
- Sialadenoma papilliferum

Cystadenoma

SOFT TISSUE TUMORS

Hemangioma

HEMATOLYMPHOID TUMORS

Hodgking's lymphoma

Diffuse large B cell lymphoma

Extranodal marginal zone B cell lymphoma

SECONDARY TUMORS

ANNEXURE – IV

STAINING TECHNIQUES.

HEMATOXYLIN AND EOSIN STAIN

Preparation of the solution :

Distilled water – 1000ml.

Ammonium alum – 100gm.

Haematoxylin – 5gm.

Absolute ethyl alcohol – 50ml.

Mercuric Oxide – 2.5gm.

100gm of ammonium alum dissolved in 1000ml of distilled water by heating and shaking at 60^{o}C. Add solution of 5gm haematoxylin in 50ml of absolute ethyl alcohol and bring rapidly to boil. When it begins to boil, remove from flame and add 2.5gm of mercuric oxide. Mix by swirling gently.

EOSIN STAIN

Eosin Y – 1gm.

Distilled water – 20ml.

95% ethanol – 80ml.

Glacial acetic acid – 0.2ml.

Dissolve 1gm eosin Y in 20ml of distilled water and add 80ml of 95% ethanol and 0.2ml of glacial acetic acid.

PROCEDURE

Bring sections to water.

Harris's hematoxylin for 15 minutes.

Rinse in tap water.

Differentiate in 1% acid alcohol – 3 to 4 quick dips.

Rinse in tap water.

Blue by placing in scott's tap water until the section appear blue.

Wash in running tap water for 10 -20 minutes.

Stain with eosin for 15 seconds to 2 minutes depending on the age of the eosin.

Rinse in tap water.

Dehydrate in 95% alcohol.

Absolute alcohol – at least 2 changes.

Xylene – 2 changes.

Mount in DPX mountant.

RESULTS :

Nuclei – Blue.

Cytoplasm, RBC, Keratin – Pink.

Eosinophilic granules – Orange Red.

MASSON'S TRICHROME STAIN (MTS)

TECHNIQUE

1. Section to water.
2. Stain in celesatine blue for 5 minutes.
3. Rinse in water.
4. Stain in meyer's haemalum for 5-10 minutes.
5. Wash in running tap water for 10 minutes.
6. Rinse in distilled water.
7. Stain in acid fuchsin solution for 5 minutes.
8. Rinse in distilled water.
9. Differentiate and mordant in a koplin jar containing 1% phosphormolybdic acid.
10. Without rinsing place slide on a cold staining rack and add just enough aniline blue solution to cover section for 5 minutes.
11. Rinse in distilled water.
12. Differentiate in aqueous 1 percent acetic acid to remove excess aniline blue.
13. Treat section in alcohol 1 percent acetic acid for 30 seconds.
14. Dehydrate in absolute alcohl.
15. Clear and mount.

RESULT:

Nuclei-blue to black.

Cytoplasm-red.

Collagen fibres-blue(or green0

Mucin-blue(or green).

PERIODIC ACID SCHIFF TECHNIQUE

SOLUTION REQUIRED:

A) 0.5% PERIODIC ACID

periodic acid -0.5gm

distilled water-100ml.

B) SCHIFF'S REAGENT

Basic fuschin-1gm

Sodium metabisulphite ,anhydrous 1gm

Distilled water-200ml

N/1 hydrochloric acid 20ml

Boil the distilled water; Add basic fuchsin and stir, cool to 50ºC.then filter and add hydrochloric acid, cool to25ºC and add Sodium metabisulphite. This solution is ready for use when it become nearly colour less,which may take two days in the dark.(Alternately activated charcoal may be added to the solution,shaken and filtered.).If the solution become recoloured it should be discarded.

TECHNIQUE:

1.Section to water.

2.Periodic acid 0.5% for 5 minutes.

3.Rinse in distilled water.

4.Schiff's reagent for 15 minutes.

5.Rinse in the three fresh changes of sulphuric acid for 2 minutes.

6.Wash in running tap water for 5 minutes.

7.Counter stain in mayer's haemalum for 30 seconds.

8.Wash in running tapwater for 5 minutes.

9.Dehydrate,clear and mount.

RESULTS:

Positive material; Magenta colour.

Nuclei: Faint grey.

ANNEXURE-V

IMMUNOHISTOCHEMISTRY

PREPARATION OF SOLUTIONS:

Citrate buffer solution- antigen retrieval solution

Trisodium citrate: 2.94 gm

1 N Hydrochloric acid: 5ml

Distilled water: 1000 ml

Required pH is 6.0, which are obtained by titration with 1N HCl.

Tris Buffer Saline (TBS) - wash buffer

Sodium chloride: 8gms

Tris (hydroxymethylamine): 0.605gms

1 N Hydrochloric acid: 4 ml

Distilled water: 1 litre

Required pH is 7.6, which are obtained by titration with 1N HCl.

Preparation of chrom alum coated slides:

Potassium dichromate: 50 mgs

Gelatin: 300 mgs

Distilled water: 100 ml

Potassium dichromate is added to distilled water and then boiled to 60◦C. Gelatin is then added slowly to it. Glass slides are then dipped in this solution and dried overnight.

After taking the required sections onto the coated slides, it is baked overnight at 45°C in the autoclave. The next day, the slides are taken for the procedure.

ANTIGEN RETRIEVAL:

The slides are arranged in a metal racket and placed in citrate buffer inside the pressure cooker, and allowed to boil up to three whistles.

Procedure:

1. Dewax the section in xylene(15 minutes each, 2 changes) and then in decreasing grades of alcohol then finally bring the sections to running tap water followed by distilled water.
2. Antigen retrieval using TBS by pressure cooker method
3. Cool to room temperature in running tap water for 20 minutes.
4. Wash in TBS -2 changes for 5 minutes each.
5. Drain and cover the sections with peroxidise block (endogenous peroxidise blocking agent) for 15 minutes.
6. Wash in TBS -2 changes for 5 minutes each.
7. Drain and cover the tissue sections with power block for 15 minutes
8. Drain and blot the excess power block.
9. Cover the sections with the respective primary antibody for 90 minutes.
10. Wash in TBS -2 changes for 5 minutes each.
11. Drain and cover the sections with super enhancer for 30 minutes.
12. Wash in TBS -2 changes for 5 minutes each.
13. Drain and cover the tissue sections with secondary antibody (HRP-horse raddish peroxidise) for 30 minutes.
14. Wash in TBS -2 changes for 5 minutes each.
15. Drain and cover the tissue sections with **DAB** (DiaminoBenzidine) substrate buffer for 5-10 minutes (depending on the time suggested in the supplied kit)
16. Wash in distilled water, counter stained with haematoxylin, clear in xylene and mount with DPX.

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BIBILOGRAPY

1. Speight.P,barret,salivary glandtumors,oraldiseases2002;8(5)229-240

2.TPsanjeevan,joshu Elizabeth,TRsaraswathi,KRanganathan.An analysis of salivarygland lesions –an institutional study J.oral Maxillofac.path.2003,7;21-24.

3.MariaP.Foschini and Vincenzo,eusebi;Value of immunohistochemistry in diagnosis of salivary gland tumors.Pathology case reviews Nov-Dec9(6)270-275.

4.Cheerik HM salivary gland in coulson WF eds.surgical pathology,2nd edn,Vol -1, Philadelphia.JB.lippin cott company.1988;21-54.

5.nagoT,GaffeyTA,Serizawa et al, Dedifferianted adenoid cystic carcinoma.A clinico pathologic study of 6 cases.mod pathol 2003;16(12) 1265-1272.

6. RiceDH.surgery of salivary gland.New Jersy.Bc Decker.inc;1982.

7.ToshitakaNagao,Eiichi Sato,Rie Inoue,Hisashi Oshiro et al, Acta histochem cytochem 2012 oct31,45(5) 269-282.

8. channggeng yi Xue ZA Zhi.bilateral Warthins tumors in a Chinese population 1990.

9.Gray's Anatomy The anatomical basis of clinlcal practice 40th edition-8th chapter

10. Surgical pathology of Head and neck-Leon Barnes 3rd edition -10th chapter-vol 11. Douglas R gnepp Diagnostic surgical pathology of Head and neck-2nd edition 2009 Saunders Elsevier 413-562

12. Modern surgical pathology 2nd edition Wiedner , Wiess

13.christopher Fletcher 4th edition ,histopathology of tumors.2013.

14. Fernando- Martinez-Madrigal et al Histology of major salivary glands. Am J Surg Pathol 1998;13(10):879-899

15. WHO classification of Head & Neck tumours 2005 edition.

16..Serafin sanchey Gomez,Juan Manuel Maza Solana et al Int Journal of otolaryngology,Head and neck surg2013,2-215-2201 17.ShinC,KimSS,ChwalsWJ.Salivary gland choristomaof anterior chest wall.J Pediatr Surg 2000;35:1506-1507

18.Brent M Kouldelka obstructive disorder chapter 3:Surgical pathology of salivary gland Eds;Ellis GL AuclairPL ,Gnepp RD Vol 25.Philadelphia WB SaundersCo;1991;26-38.

19.Modern immunohistochemistry piguo chu, lawrence, weiss 2009-cambridge 52-77

20.DavidJ.DABBS Diagnostic Immunohistochemistry.Theranostic and genomic applications. 2010. Elsevier .page 259-280.

21.Davies JNP ,Doge OG .,Burkitt DP.1964 salivary gland tumors in Uganda.Cancer,17:1310-1322.

22.Juhan rosai &AckermanCHAPTER 21 page 1805.10th edition.

23.Arruda Morias ,Paulo Roberto A zevedo,Carvalho,Medeiros,Lajus and Lopes Costa.clinicopathological study of salivary gland tumors.An assessment of 303 patients.Cad.Saude.Publica,Rio de Janerio.2011;27[5]:1035-1040.

24.Gnepp DR &EL –Mofty SK.Salivary gland,chapter51,ANDERSONS Pathology

25.Francis Marchal,Pavel Dulguerov,Sialolithiasis management.Arch.otolaryngol Head &neck surgery.2003;129:951-956.

26.Hoffman DA,Mc Conahey WM ,FraumeniJF ,et al.cancer Incidence following treatment of hyperthyroidism.Int J Epidemiol1982;11(3)218-224.

27.Whatley WS,Thompson JW, Rao B.Salivary gland tumors in survivors of childhood cancer.Otolaryngol Head &Neck Surg 2006;134(3):385-388.

28.Sadetzki S, Chetrit A ,Jarus-Hakak, et al.Cellular phone use and risk of benign and malignant parotid tumor- a national widecase-control study .Am J Epidemiol 2008;167(4):457-467.

29.GrahamS,Blanchet M ,Rohrer T.Cancer in asbestos –mining and other areas of Quebec.J Natl Cancer Inst 1977;59(4)1139-1145.Int J Cancer1996;67(2)194-198.

30.ZhengW, Shu XO.Ji BT et al.Diet and other risk factors for cancer of salivary glands;apopulation –based case control study.Int J Cancer1996;67(2)194-198

31.Glas AS,Hollema H,Nap RE,et al.expression of estrogen receptors,progesterone receptors and insulin like factor receptor-1 and Ki-67 in patients with recurrent pleomorphic adenoma of the parotid gland.Cancer2002;94(8):2211-2216.

32.Auclair PL, Ellis GL .Atypical features in salivary gland mixed tumors:Their relationship to malignant tranformatin .Mod Pathol1996;9(6)652-657

33.Srijon mukerji,sunith shethet al warthins tumor of bilateral tumor of parotid glands.a case report J.Maxillofac:oralsurg 11(4)483-486

34.ChungYF,Khoo ML, HingMK,et al.Epidermiology of of Warthins tumor of parotid gland in asian population. Br .J.surg1999 may86(5)661-664.

35. Foote FW Jr. Frazell EL. Tumours of the major salivary glands. Cancer 1953; 6: 1065-1133.

36. Ahlbom HE. Mucous and salivary gland tumours: Clinical study with special reference to readiotherapy based on 254 cases treated at the Radiumhemmet, Stockholm. Acta Radio 1935; 23 (Suppl): 1-452

37. Kleinsasser O, Klein H.J. Basal Zelladenomeder speicheldrusen. Arch Klin Exp Ohren - Nasen Kehlkopfheilk 1967; 189: 302-316.

38. Hildebrad O. Veber angeborne epitheliale. Cystenund Fistein des Halse. Arch F. Klin Chir 1895; 49: 167-192.

39. Brandman et al ; oncocytic tumors of major salivary glands. A study of 68 cases with follow up of 44 patient.AM J SurgPathol 1991;(15):514-528.

40.Tashitaka Nagao,Isamu Sugano,Osamu Matsuzaki,et al.intraductal papillary tumors of major salivary gland .Arch Pathol Lab Med2000;124;291-295.

41.Paul L Auclair Gary L Ellis and Douglas R Gneep other epithelial neoplasms.chapter 15 surgical pathology of salivary gland Eds:Ellis GL, Auclair PL, Gnepp RD vol 25Philadelphia WB saunders Co. 1991;252-268.

42. Paul L Auclair Gary L Ellis Mucoepidermoid carcinoma.chapter 16 surgical pathology of salivary gland Eds:Ellis GL, Auclair PL, Gnepp RD vol 25 Philadelphia WB saunders Co. 1991:269-298

43. Harry.L Evans et al mucoepidermoid carcinoma of salivary glands study of 69 cases with special attention to histologic grading – AMJ clinical pathology 1984; 81-696-701

44. Griddin et al Salivary mucoepidermoid Cacinoma of parotid gland. Arch pathology med, sep 2004, 128(9): 1046-1049.

45.Lewis JE et al Mucoepidermoid Cacinoma of parotid gland Mayo clinic experience. 2004.Arch Otolaryngol Head Neck surg(130) 849-856.

46. EllisGL, Auclair PL. Tumors of salivary gland.In AFIP Atlas of Tumor pathology,4th series.9th fascicle.Washington DC:American Registry of pathology;2008

47.CharlesE Tomich adenoid cystic carcinoma chapter 19 surgical pathology of salivary gland Eds:Ellis GL, Auclair PL, Gnepp RD vol 25 Philadelphia WB saunders Co. 1991:333-349

48.YoonJH AbnSG,et al calcification in a clear cell Mucoepidermoid carcinoma of hard palate:IntJ.Oral Maxillofac Surg 2005(34)927-929.

49.SekineJ,Anami M,fajitas,et al, A case of mucoepidermoid carcinoma with melanin pigmentation manifested in palate.virchows Arch;2005,446-462.

50.NagoT,GaffeyTA,SerizawaH,Suganol,Y,YamazakiK,TokashikiR,YoshidaT,Minato H,KayPA, LewisJE.Dedifferentiated Adenoid cystic carcinoma: a clinicopathological studyof 6 cases.Mod pathol2003;16(12):1265-1272.

51.KowalskiP J,PaulinoAF.Perineural invasion in adenoid cystic carcinoma:its causation/promotion by brain brain-derived neurotrophic factor.Human Pathol 2002sep;33(9):933-936.

52.Matilda Llupi et al:mucin expression in normal salivary gland mucoepidermoid carcinoma.A study of 42 cases of mucoepidermoid carcinoma .Department of oral pathology,odontology in Malmo;Dec;2013.

53.YokoyamaM,NomuraY,SembaT.acinic cell carcinoma of the parapharyngeal space; case. head neck1993;15(1);67-69.

54.Hwei-YeeLee,Kent Mancer,et al.Primaryacinic cell carcinoma of lung withLymph node Metastasis.Arch Pathol Lab Med.2003;(127)216-219.

55.Henley JD,RehanJ,Oda Det al.Metaplastic tumors of salivary gland. Origin.Oral surg Oral Med Oral Pathol Oral Radiol Endod 1977;8:188-197.

56. Duvvuri U et al 2012 DOG1 : novel marker of salivary acinar and intercalated duct differentiation. Mod pathology 25:919-924.

57. Simpson RH et al:1997, well differentiated acinar cell carcinoma of salivary glands associated lymphoid stroma. Hum pathology;28:595-600

58.Luna et al MA et al PLGA A study of 40 cases with long term follow up and an evaluation of morphology of papillary areas.AM J Surg Pathol;2000: 24;1319-1328.

59: Wceiuk & JKC chan et al, advances in salevary lavel histopathology 2007, 51, 1-20

60. Matila lupi Exp of muceins in noumal salivary glands and nucopladermoid carcinoma of salivary glands. Malmo university. 2013 1-17

61. T. Hamada et al: Mucin expression in plenoepluc Adunoma of salivary gland. A potential role for MUC_1 as a maker to predict recurrence. Jclin pathol, 2004 August 57 (8); 813- 821.

62: Vacchi – suzzim, Boccielicle, et al ki-67 proliferation rate as a proguostic maker in major salivary gland carcinomas; Ann otol Rhivol laryngol 2010- oct 119(10) 677-83.

63. Inmmunoletoclunicial aspects in pleounopic advoma related to its histiogensis and malgiuitaion Mioara Trandfriresl etal. Romanian Journal of oral Reliation vol4, No.4october –December 2012.

64. P. Deihimy DDS, et al; study of alypeptiheal cell markers in pleourplic Aderoma and mulipeider mold carcionoma of salivary glands. Dental Research Journal (Vol 3, No.2 Actumn – winter 2006.
65. Toshitaka Nagao, Eiichi sato et al Immoulentoclimical analysis of salivary glands Thomors. Application for surgical patheology practial. Application for surgical pathology pratical Acta Histocleum cytoclem. 2012 oct 31, 45 (5) : 269-282.
66. Auca – Stegania Contributions to the histoclmical study of plenoporic Adnome of major salivary glands. GRaiva 2013. 1-14.
67. Heiklai Luukka. Salivary gland cancer in Finland . Incidence, Histological distribution outcome and prognostic factors. Turn ytlipisto 2010-1-84.
68. Salivary duct carcinoma; Immunhisto climical profile of an aggressive salivary gland tamor A. Etges, D.S pinto etal J. Clin pathol 2003, December 56(12) 914-918.
69. CL. Margritescu et al Tumoral stoma of salivary pleounprphic adeuoma histopathological histochemiacal and immohistochmical study. Romanian journal of mprphology and Embrology 2005, 46(3), 211-223.
70. Shin – ichur et al. differential expression of p 63 isotypes in salivary gland neplasms; biological and diagnostic implications. Hum pathol 36, 821- 827.
71. Fosehini et al p63, expression is salivary gland tumos; role of Delta NP 73L in neoplastic transformation Int J. Surg pathol 13, 329-335.
72. Edwards pc, Bhuiya TEtal, Assessment of p 63 expression in salivary. Gland neiplasn aduovid cystic carcinoma. Polymorphous locu grade adeno carcinoma and basal all and canalicular adenuoma. Oral sarg oral mod oral pathol oral Radiol Endol 97, 613- 619.
73. Weber A, et al Expression profiles of p 53, 0 63 and p73 leugin salivary gland tumors virchols arch 2002, Nov 441(5) 428-36
74. Mc Hugh JB et al p 63 immunhistochemistry differtiatls salivary gland on cotoma and oncoafic carcinoma flom metastatic rural cell carcinoma Head Neck pathol : 2007 Dec 1 (2) 123-31.
75. Bilal H, Handra Luca A. P 63 is expressed in basal and nupotherlical cells of human normal and tumor salivary gland tissues. Histochem cytochem. 2003, Feb 51(2) 133-9.
76. Little NA, et al P 63 Int J. Biochem cell Biol 2002 Jan: 34(1) 6-9

77. Moll um et al p 63 and p 73, role in denelopment and tumor formation mol cancer Res. 2004 Jal 2 (7) 371-86.

78. Hdst VA, Marshall CE, et al KIF protein expression and analysis of c-kit gene mutation in adeuoid cystic carcinoma. Modern pathology Inc (!999, 12 (10), 956-960)

79. Chriisopler A moskaluic et al C- kit gene mutation in adevoid cystic carcinoma are rare. Modern pathology (2010) 23, 905-906.

80. Carla R penner et all C- Kit Expression Disinguishes Salivary gland Adeniod cystic carcinoma from polymorplues low-grade Adeno carcinoma mod. Pathol 2002, 15 (7) 687-691.

81. Martineing Barbae et al Salivary duct carcinoma clinicopathological and immuno histochemis studies. J craniomaxillo fac surg 1997 Dec, 25 (6) 328-34.

82.NagoT,Gaffey TA,Serizawa H et al.sarcomatoid variant of salivary duct carcinoma;A distinct histopathological and immunohistochemical study of eight cases with review of literature.Am J .clin.pathol.2004:122;222-231.

83.Ide F ,Mishima K ,et al.Sarcomatoid Salivary duct carcinoma of oral cavity.Virchows Arch.2003;443;686-689.

84.Simpson RH,Prasad AR et al Mucin rich variant of salivary duct carcinoma:A clinicopathological and immuhistochemical study of four cases.Am.J .Surg. Pathol. 2003;27;1070-1079.

85.NagoT,Gaffey TA,Visscher DW et al.invasive micropapillary salivary duct carcinoma;A distinct histological variant with biological significance .Am J .clin.pathol2004;28;319-326.

86.Fan C Y et al 2000 Expression of androgen receptor and prostate specific marker in salivary duct carcinoma. An immmunohistochemical analysis of 13 cases and review of literature.A m j Surg Pathol 24;579-586.

87.Laforga J B 2004 Salivary duct carcinoma with neuro endocrine features.Virchows Archs 444:473-476.

88.Brandwein M S et al 1990 Salivary duct carcinoma. A clinicopathological and immune histochemical study of 12 cases. Cancer 65;2307-2314.

89. Leivo I Jee K J et al 2005 characterization of gene expression in major type of salivary gland carcinoma with epithelial differentiation.Cancer Genet Cytogenet156;104-113.

90. Cheuk W ,M iliauskas et al 2004 ,intra ductal carcinoma of oral cavity:a case report and a reappraisal of the concept of the concept of pure ductal carcinoma in situ in salivary duct carcinoma.Am J Surg Pathol28;266-270.

91.Jayakrishnan A Elmalah et al 2003 Basal cell adenocarcinoma in minor salivary glands.Histopathology42:610-614.

92. Sharma et al Basal cell adenocarcinoma:Report of a case affecting the submandibular gland.J Oral Maxillofac Pathol July-Dec 2007;11(2):56-59.

93.Ellis GL, Auclair PL, GneppDR, et al other malignant epithelial neoplasms.In: Ellis GL, Auclair PL, Gnepp RD,eds,surgical pathology of salivary glands. Philadelphia WB saunders Co. 1991:471-476.

94.HuYH,et al.Abberent protein expression andpromotor methylation of p16 gene are correlated with malignant transformation of salivary pleomorphic adenoma.Arch pathol LabMed2011.135(7)882-889.

95.Bassel Tarakjiet al, Immunohistochemical Expression of p53 in Pleomorphic Adenoma and Carcinoma Ex Pleomorphic Adenoma Journal of Cancer Epidemiology Volume 2010 (2010).

96.Eiji Mitate, Shintaro Kawano, Tamotsu Kiyoshima, Toshiyuki Kawazu, Toru Chikui, Yuichi Goto,Ryota Matsubara, Seiji Nakamura. Carcinoma ex pleomorphic adenoma of the upper lip: a case of an unusual malignant component of squamous cell carcinoma.World J Surg Oncol 2013 17;11:234.

97.QureshiA A,et al 1994 Benign metastasizing pleomorphic adenoma.A case report and review of literature. Clin Orthop Rel Res 308;192-198.

98.Hui KK, Luna MA et al, undifferentiated carcinomas of major salivary glands.Oral Surg.Oral Med.Oralpathol1990;69;76-83.

99.NagoT,Gaffey TA,Serizawa H et al. small cell carcinoma of major salivary glands:clinicopathological study wit eight cases with review of literature.Am J .clin.h emphasis on CK -20 immunoreactivity and clinical outcome. .Am J Surg pathol.2004;128;7622-770

100.Chan JK ,Suster Set al, cytokeratin 20 immunoreactivity distinguish Merkel Cell carcinoma and salivary gland small carcinomas from small cell carcinomaof various sites. .Am J Surg pathol.1997;21;226-234.

101.WorleyN K ,et al, lymphoepithelial carcinoma of minor salivary gland.Arch Otolaryngol Head Neck Surg 123;638-6 .

102.Ajay Kumar Bansal ,Ruchi Bindal,Charu Kapoor,Sharad Vaidya and Harkanwal Preet Singh current concept in diagnosis of unusual salivary gland tumors.DentRes J (Isfahan).dec 2012;9(suppl):S9-S19.

103.Ying Y L,et al,squamous cell carcinoma of parotid gland.Head Neck2006,28(7):626-632.

Chandan V S et al Is c-kit (CD-117) immunolocalization of in cell block preparation useful in the differentiation of adenoid cystic from pleomorphic adenoma? Cancer 2004;102;207 -209.

104.Seethala RR,et al .CD-43 expression in adenoid cystic carcinoma.Mod. Pathol.2004;17(suppl);232A.

105.Handra –Luca A,et al.MUC1,MUC2,MUC4,MUC5AC expression in salivary gland mucoepidermoid carcinoma;diagnostic and prognostic implications. Am J Surg pathol.;2005;29881-889.

106.Suzuki M ,IchimiyaI et al. Histopathological features and prognosis of patient wit h mucoepidermoid carcinomaof parotid gland.J.Laryngol.otol.1998:112;944-947.

107.Ryuichi M urase et al Novel therapeutics strategies for malignant salivary gland tumors;Lessons learned from breast cancer.International journal of oto laryngology,vol 2011,1-9.

108.Vuhahula EAM Salivary gland tumors in Uganda.A clinicopathological study .Africian health sciences2004;4(1):15-23.

109.Ito FA ,K Ito ,PA Vargas,O P de Almeid MA Lopes . salivary gland tumors in a Brazilian population:a retrospective study of 496 cases.International J of Oral and Maxillofacial Surg.2005:34[5]:533-536.

110.Thomas KM,HuttMSR,Borginstein salivary gland tumors in Malawi.Cancer 1980;46:2328-2334.

111.Otoh EC,Silas OA, Echejoh GO,Menasseh AN, Mandog BM . Descriptive pattern of salivary tumors.A10-year retrospective study.Annals of Africian Medicine July-September2009;8:199-202 In Jos University Teaching Hospital .

112. Felipe Paiva Fonseca, ,A Marianne De Vasconcelos Carvalho, A

Oslei Paes De Almeida, clinicopathologic Analysis Of 493 Cases Of Salivary Gland

Tumors In A Southern Brazilian Population vol.114 No 2 August 2012

113.Jones AV ,CraigGT,Speight PM,FranklinCD. The range and demographies of salivary

gland tumors diagnosed in a U.K.Population.Oral Oncology.2008;44[4]:407-417.

114.Gupta SK,Sengukpta P,Sarkar SK .1975.Primary tumors of salivary glands.JI nd MedAssoc,65[10]:277-280

115.Krishnaraj Subhasraj ,Salivary gland tumors: A single institution experience in India. British Journal of Oral and Maxillofacial surgery 2008:46:635-638.

116.Ahmad S,Lateef M,Ahmad R. clinicopathological study of Primary Salivary Gland Tumors in Kashmir .JK-Practioner.2002;9[4]:231-233.

117.Rewsuwan ,Jonglolnee Settakom,Pongsak Mahanupab. Salivary gland tumors in maharaj Nakorn Chiang Mai hospital:ARetrospective study of 198 cases.Chaing Mai Med Bull.2006:45[2];45-53.

118.Satko I,Stanko P and LongauerovaI. Salivary gland tumors treated in the stomatological clinics in Bratislava.Craniomaxillofac Surg 2000;28[1]:56-61.

119Mohammed Ayub M,Zahid Sohail,Abbas Zafar and Shoukat Malik. Morphological patterns of parotid tumors.Journal of the college of the Physicians and Surgeons.2008;18[5];274-277.

120.Buddhraj SN, Pasupathy,Perianayagam.Salivary gland tumors in Pondicherry.Ind J Sur1974;36:235-239.

121.Morgan MN ,Mackenzie DH. tumors ofsalivary glands.A review of 204 cases with 5 year follow up Br J Surg 1968;55[4];284-288

122.Jasani B ,Schmid K.W,Immunocytochemistry in diagnostic histopathology,Churchill Livingstone,Edinburg,1993,32-135.

123. Hashimoto, K., Yamamoto, H., Shiratsuchi, H., Nakashima, T.,Tamiya, S., Higaki, Y., Komune, S., Tsuneyoshi, M. and Oda, Y.(2011) S100P expression in ductal type of carcinoma ex pleomorphicadenoma. Am. J. Surg. Pathol. 35; 346–355.

122.Arruda Morias ,Paulo Roberto A zevedo,Carvalho,Medeiros,Lajus and Lopes Costa.clinicopathological study of salivary gland tumors.An assessment of 303 patients.Cad.Saude.Publica,Rio de Janerio.2011;27[5]:1035-1040.

123.Davies JNP ,Doge OG .,Burkitt DP.1964 salivary gland tumors in Uganda.Cancer,17:1310-1322.

124.Chatterjee and Panda .A pathological study of benign and malignant tumors of salivary glands.

125.Skolnik EM.1977.Tumors of major salivary gland.Laryngoscope,84:843-861.

126.Sharkey FE.Clinicopathologic study of 366 salivary gland tumors.Am J Clin Pathol1977,67;272-278.

127.Renehan A,GleaveEN,MancockBD,SmithP,McGurk M .long term follow up of over 1000 patients with salivary gland tumors treated in a single centre.Br J Surg 1996;83:1750-1754.

128.Nagarkar NM., BansalS DassA ,Singhal SK,Mohan H.salivary gland tumors-our experience. Indian Journal of Otolaryngology,Head and Neck Surgery. Jan-Mar2004:56(1):31-34.

129.KhazanchiRK,SahaSS,DeepakMittal,Rana Patir,Dhawan IK, Tumors of parotid gland .A Review of 88 patients of current methods of treatment.Indian Journal of cancer.1988:25;1-6.

130.Dardick L ThomasM J et al Myoepithelioma new concepts of histology and classification ;a light and electron microscopic study.Ultra struct Pathol 13:187-224.

131.Vargas PA ,Rene Gerhard,J F Araujo and Vieira de castro.Salivary gland tumors in Brazilian population .A retrospective study of 124 cases.Rev Hos Clin Fac Med S Paulo2002;57[6]:271-276.

132.Agarwal RV,Solanki BR,JunnarkarRV 1967 Salivary gland tumors.Ind J Cancer,4:209-213.

133.Ochicha O ,MalamiS,Mohammed A,Atanda A .A histopathological study of salivary gland tumors in Kano,northern Nigeria.Indian JPathol Microbiol 2009;52:473-476

134.Evans RW and Cruickshank AH.Epithelial tumors of the salivary glands.Vol1.Philadelphia:WB saunders Co .,1970.

135.Mag A. S Coutulbea,S.lupescu,H.stefanescu,C. doros and A.ruja.Parotid gland tumors..journal of experimental medical and surgical research.2010;17[4]:259-263.

136.Leon Barnes and susan Muller.Basal cell adenocarcinoma of the salivary glands.cancer.1996;78:2471-2477

137.Nikitakis NG,,TosiosKI,PapanikolaouVS,et alImmunohistochemical expression of cytokeratin 7 and 20in malignant salivary gland tumors.Mod Pathol 2004;17[4];407-415.

138.Sadayuki Kaba,Masaru Kojima,H azuki Matsuda,Shiro Sugihara,N masawa,K.Kobayashi and Toshio Fukudo.Kuttners tumor of the submandibular glands.diagnostic cytopathology.2006;34;631-635.

139.Wah Cheuk and chan.Kuttner of the submandibular gland.A m J Clin Pathol 2002;117:103-108.

140.Anna Kazanceva,Valeria,Groma,Leine smane,EgilisKornevs and Uldis Teibe.proliferative potential in benign mixed salivary gland tumors.An assessement of 303 patients.Cad.Saude Publica,Rio de Janerio.2011;27[5];1035-1040.

141.Rasp G et al, Malignant salivary gland tumors: Squamous cell carcinoma of the submandibular gland in a child. Am J otolaryngol 1992; 13: 109 -112..

142.Seifert G., Donah K: Classification of pathohistology of disease of the salivary glands: Review of 2,600 cases in the salivary gland register. Berichte Path Bd. 1976; 159: 1-32.

143. Regezi JA, Zarbo RJ, Batsakis JG:Immunoprofile of mucoepidermoid carcinomas of minor salivary glands. OralSurg Oral Med Oral Pathol 1991; 71: 189-192.

144.Loyola AM, Sousa SO, Araujo NC, AraujoVC. Study of minor salivary glandmucoepidermoid carcinoma differentiation based on immunohistochemical expression of cytokeratins, vimentin and muscle specific actin. Oral Oncology 1998; 34(2):112-18.

145.Foschini MP, Marucci G, Eusebi V: Lowgrade mucoepidermoid carcinoma ofsalivary glands: characteristicimmunohistochemical profile and evidence of striated duct differentiation. VirchowsArch. 2002 May;440(5):536-42.

146.E dward P .C.,BhuiyaT,.Kelsch R.D.[2004].Assessement of P63 in the salivary gland neoplasms adenoid cystic carcinoma ,polymorphous low -grade adenocarcinoma and basal cell and canicular adenoma . . OralSurg Oral Med Oral Pathol Oral Radiol Endod 97,613-619.

147.DardikI,Ostrynski V.L.,EkemJ.K.,Leung R.,B urford –Mason A.P.[1992] Immunohistochemical and ultra structural correlates of muscle-actin expression in pleomorphic adenoma and myoepithelioma based on formalin and methanol fixation.Virchows Arch A Pathol Anat Histopathol 421,95-104.

148.Press M.F.,Pike M..,Hung G.,(1994).Amplification and overexpression of HER-2/neu in carcinoma of the salivary gland:correlation with poor prognosis.cancer Res54,5675-5682.

149.Chen J.C.,Gnepp D.R.,Bedrossian C.W.1988.adenoid cystic analysis of adenoid cystic carcinoma of salivary glands:an immunohistochemical analysis. . OralSurg Oral Med Oral Pathol65,316-326.

150.Holst V.A.,Marshall C.E.,Moskaluk C.A.,Frierson H.F.,Jr.[1999] KIT protein expression and analysis of c-kit gene mutation in adenoid cystic carcinoma.Mod Pathol 12,956-960.

151.Amoueian,S Saghafi,F.Farhadi,E.Tohidi and L.Sa egi I mmunohistochemical Assessment of Ki-67 expression in adenoid cystic carcinoma of the salivary gland.Iranian Journal of Basic Medical Sciences.2007;10[2]:84-89.

152.Nagao, T., Sugano, I., Ishida, Y., Hasegawa, M., Matsuzaki, O.,Konno, A.,Kondo, Y. and Nagao, K. (1998) Basal cell adenocarcinoma of the salivary glands: comparison with basal cell adenoma through assessment of cell proliferation, apoptosis, and expression of p53 and bcl-2. Cancer 82; 439–447

153.Jaehne, M., Roeser, K., Jaekel, T., Schepers, J. D., Albert, N. andLöning, T. (2005) Clinical and immunohistologic typing of salivaryduct carcinoma: a report of 50 cases. Cancer 103; 2526–2533.

CHART 1

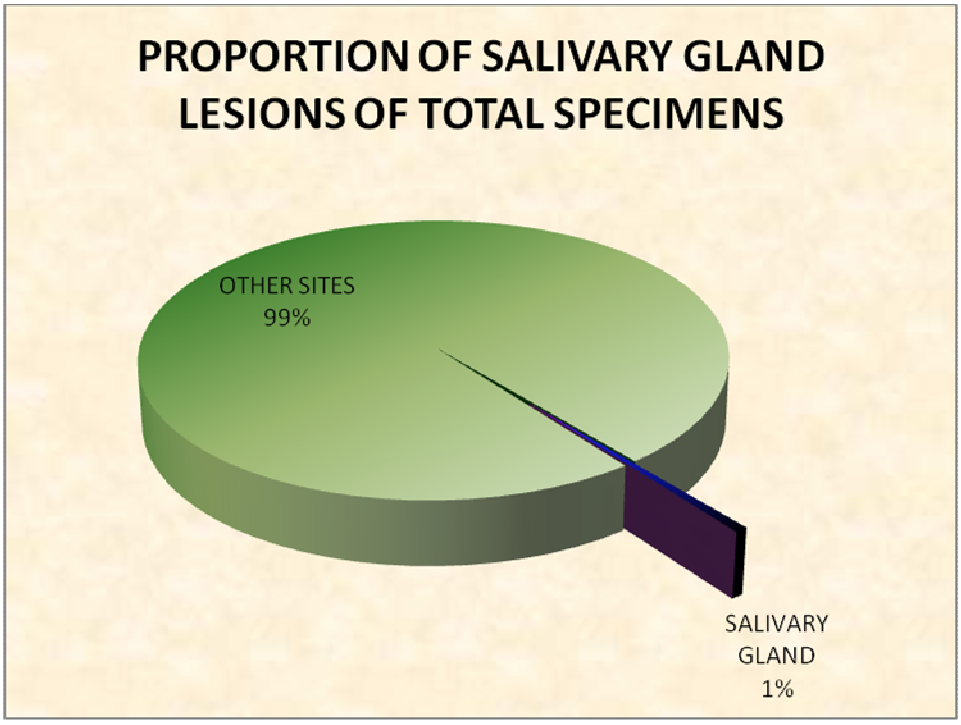
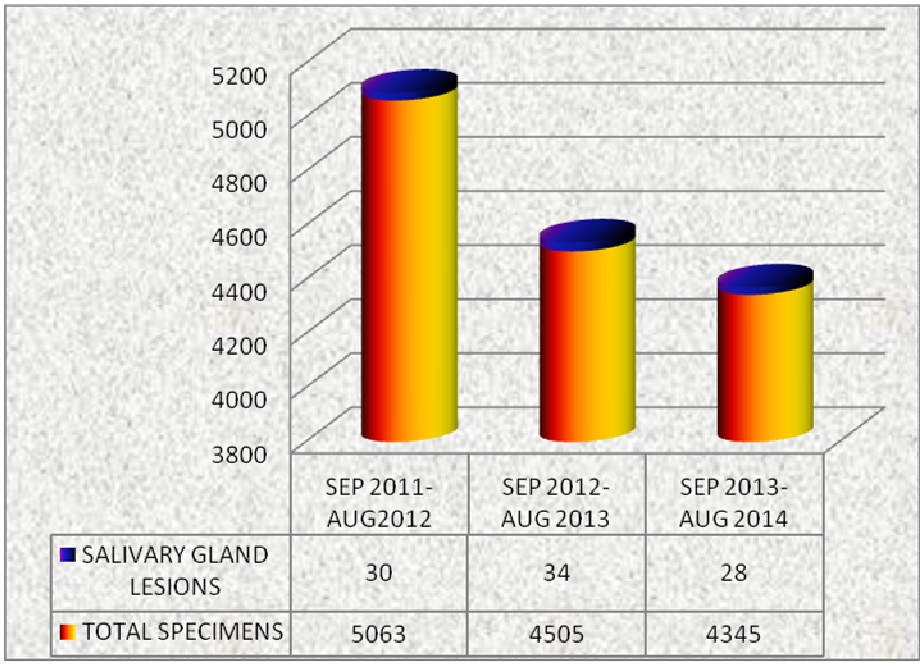


CHART 2

INCIDENCE OF SALIVARY GLAND LESIONS



	SEP 2011-AUG2012	SEP 2012-AUG 2013	SEP 2013-AUG 2014
SALIVARY GLAND LESIONS	30	34	28
TOTAL SPECIMENS	5063	4505	4345

CHART 3

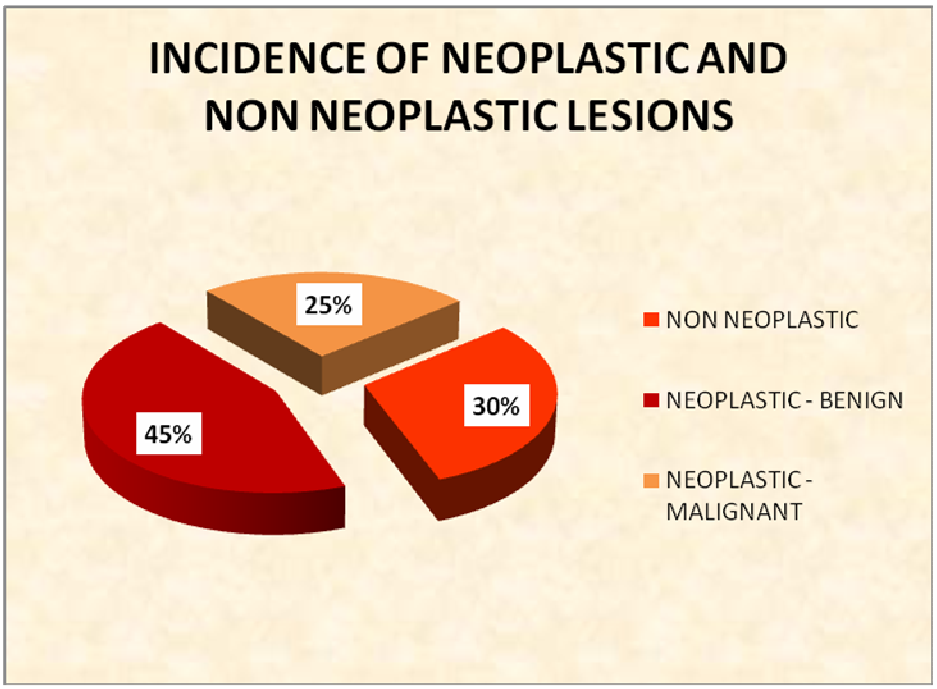
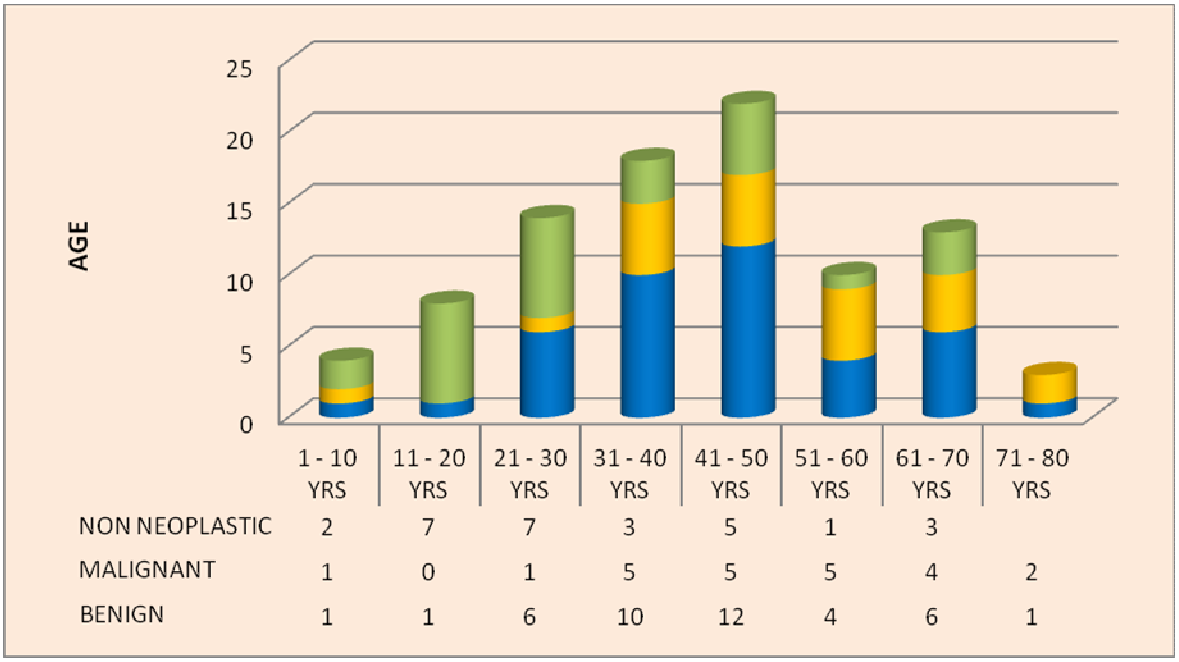


CHART 4

AGE DISTRIBUTION OF SALIVARY GLAND LESIONS



	1 - 10 YRS	11 - 20 YRS	21 - 30 YRS	31 - 40 YRS	41 - 50 YRS	51 - 60 YRS	61 - 70 YRS	71 - 80 YRS
NON NEOPLASTIC	2	7	7	3	5	1	3	
MALIGNANT	1	0	1	5	5	5	4	2
BENIGN	1	1	6	10	12	4	6	1

CHART 5

SEX DISTRIBUTION OF SALIVARY GLAND LESIONS

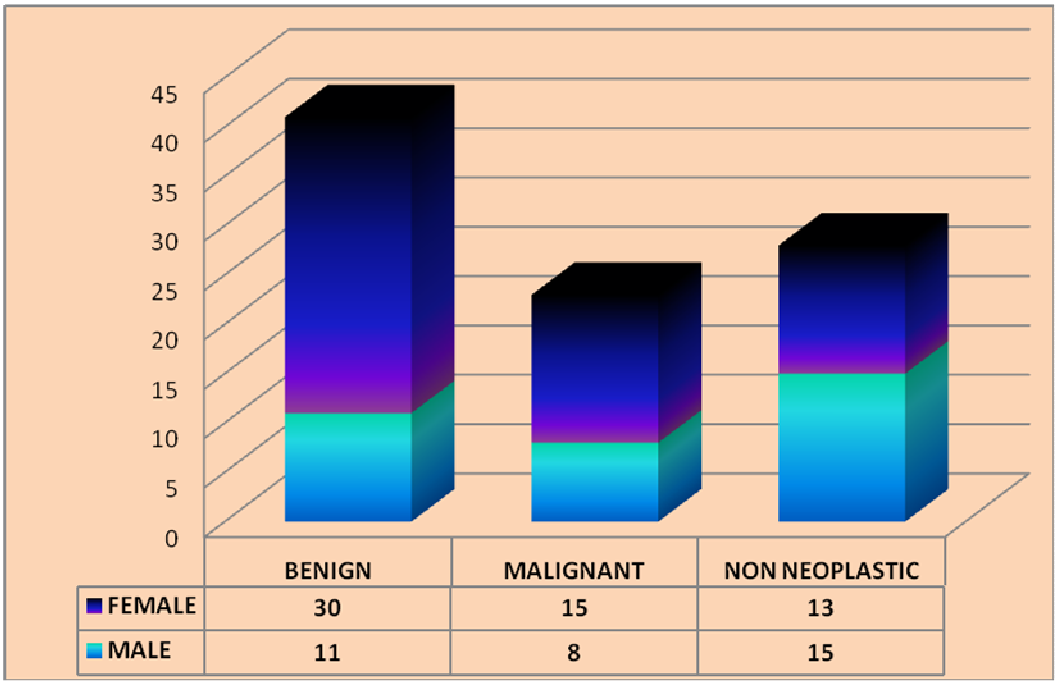


CHART 6

SITE DISTRIBUTION OF SALIVARY GLAND LESIONS

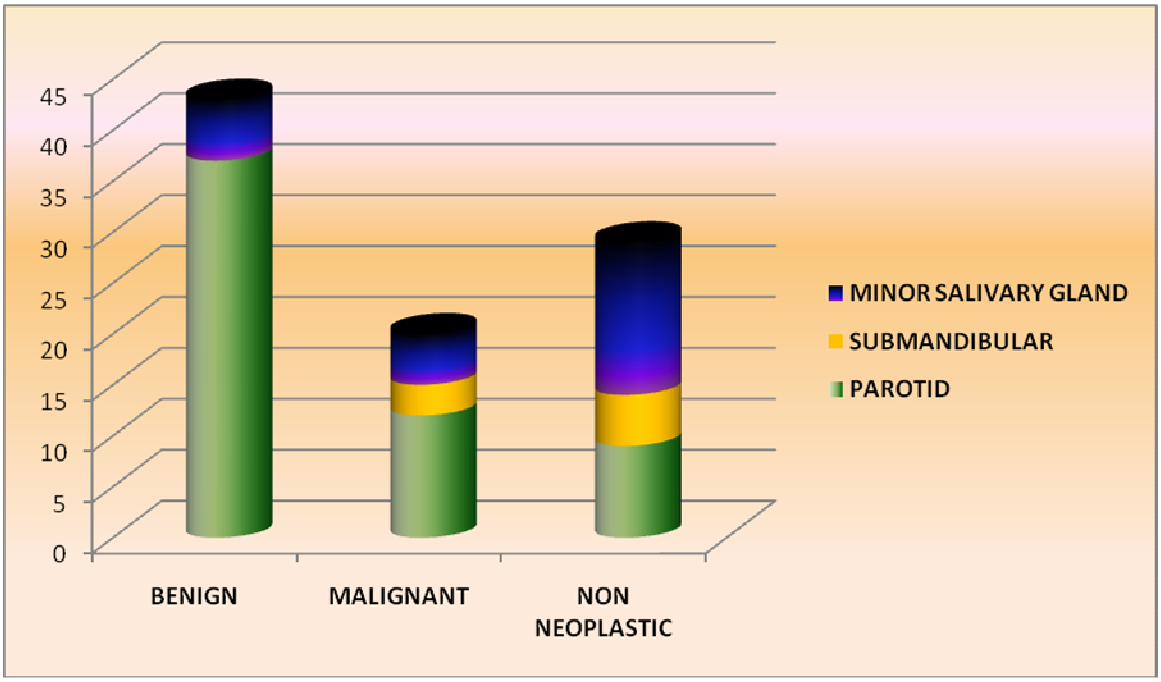
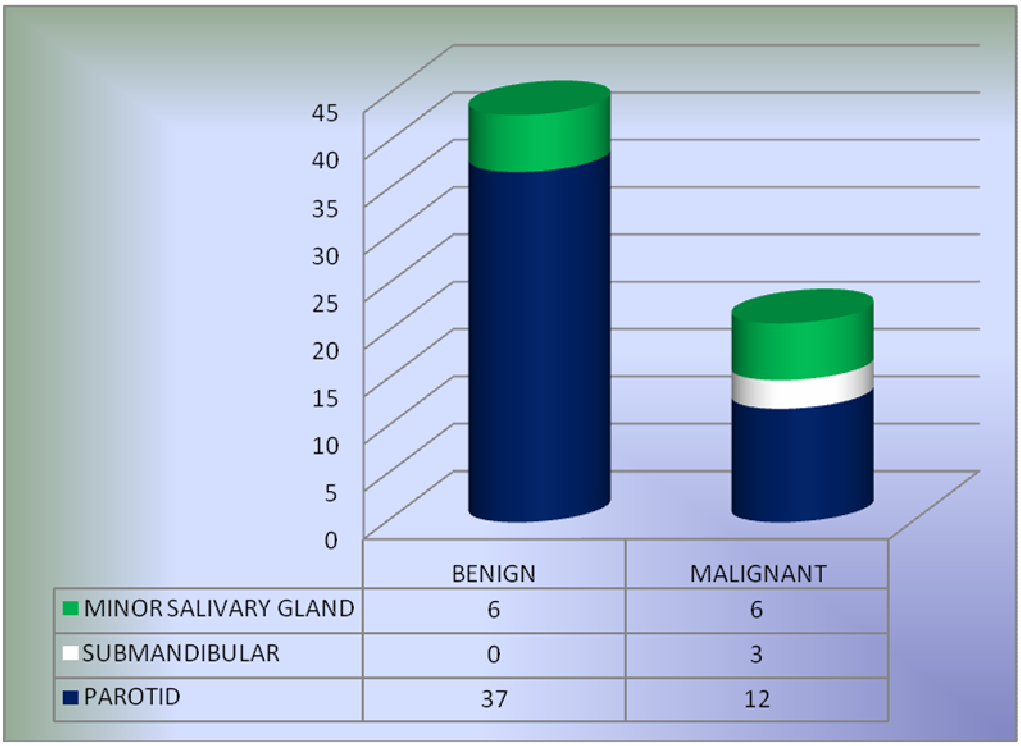


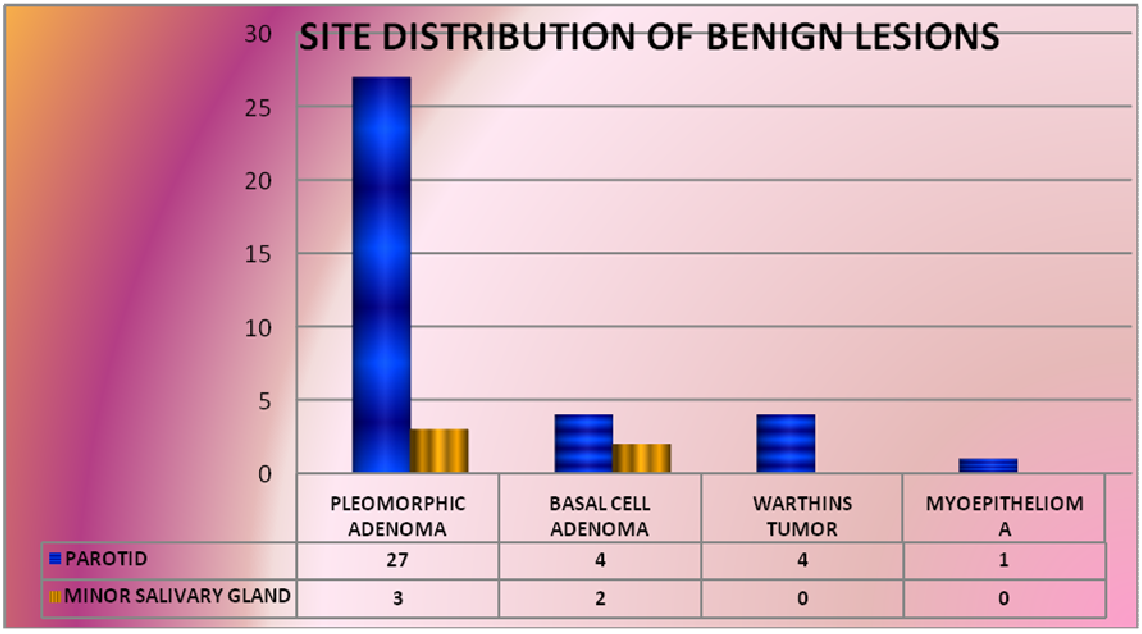
CHART 7

SITE DISTRIBUTION OF SALIVARY GLAND TUMOURS



	BENIGN	MALIGNANT
MINOR SALIVARY GLAND	6	6
SUBMANDIBULAR	0	3
PAROTID	37	12

CHART 8



	PLEOMORPHIC ADENOMA	BASAL CELL ADENOMA	WARTHINS TUMOR	MYOEPITHELIOMA
PAROTID	27	4	4	1
MINOR SALIVARY GLAND	3	2	0	0

CHART 9

SEX DISTRIBUTION OF MALIGNANT TUMOURS LESIONS

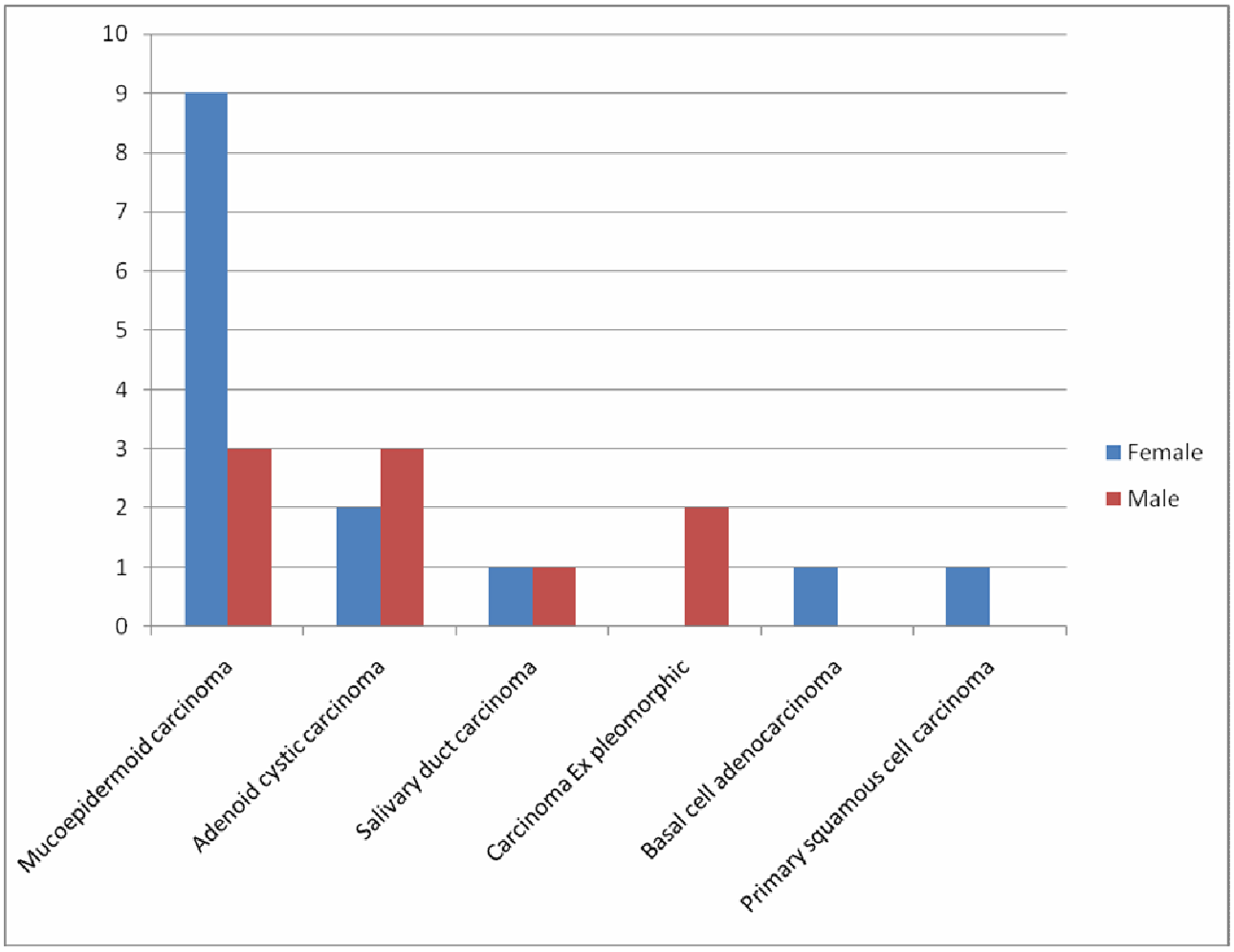
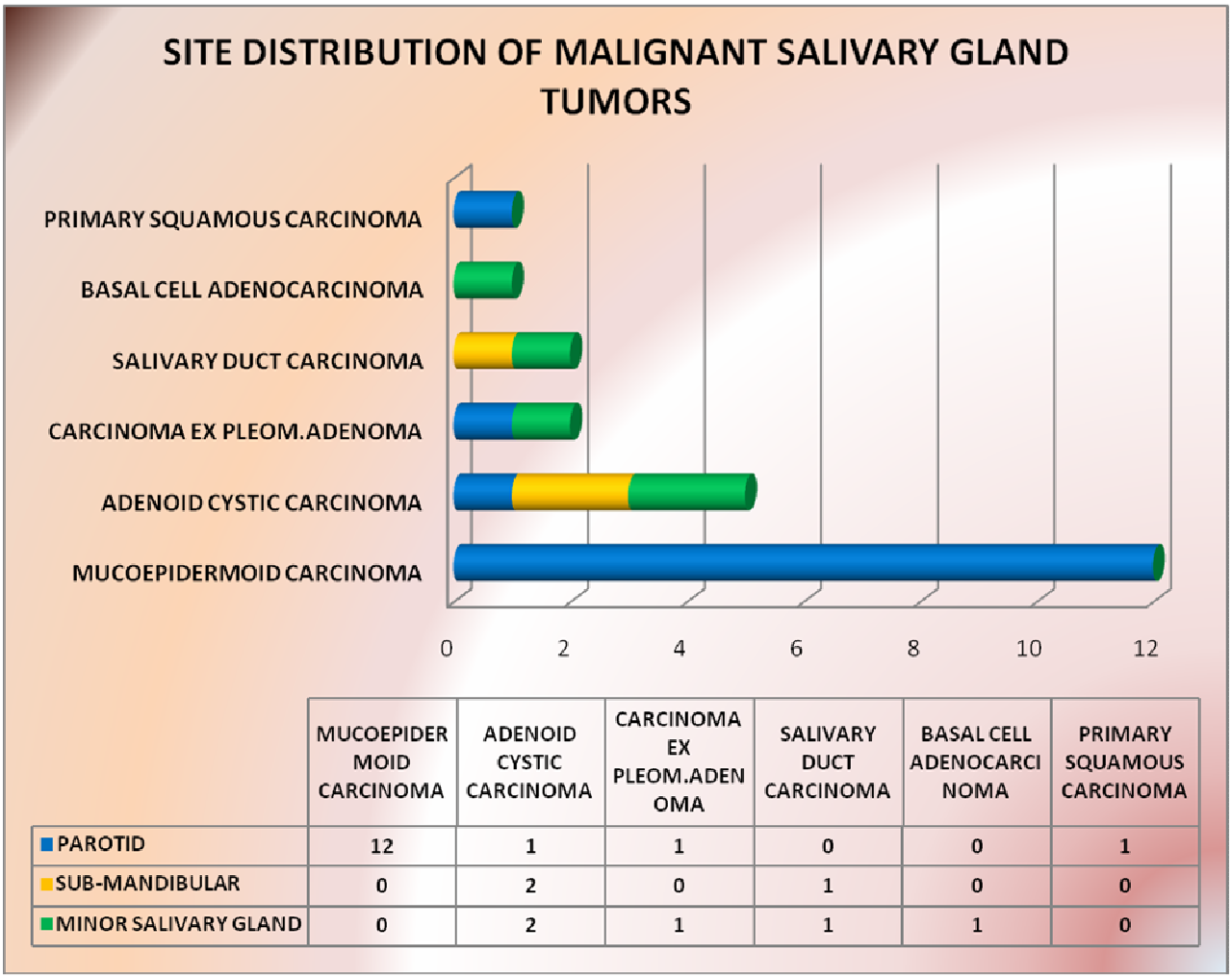
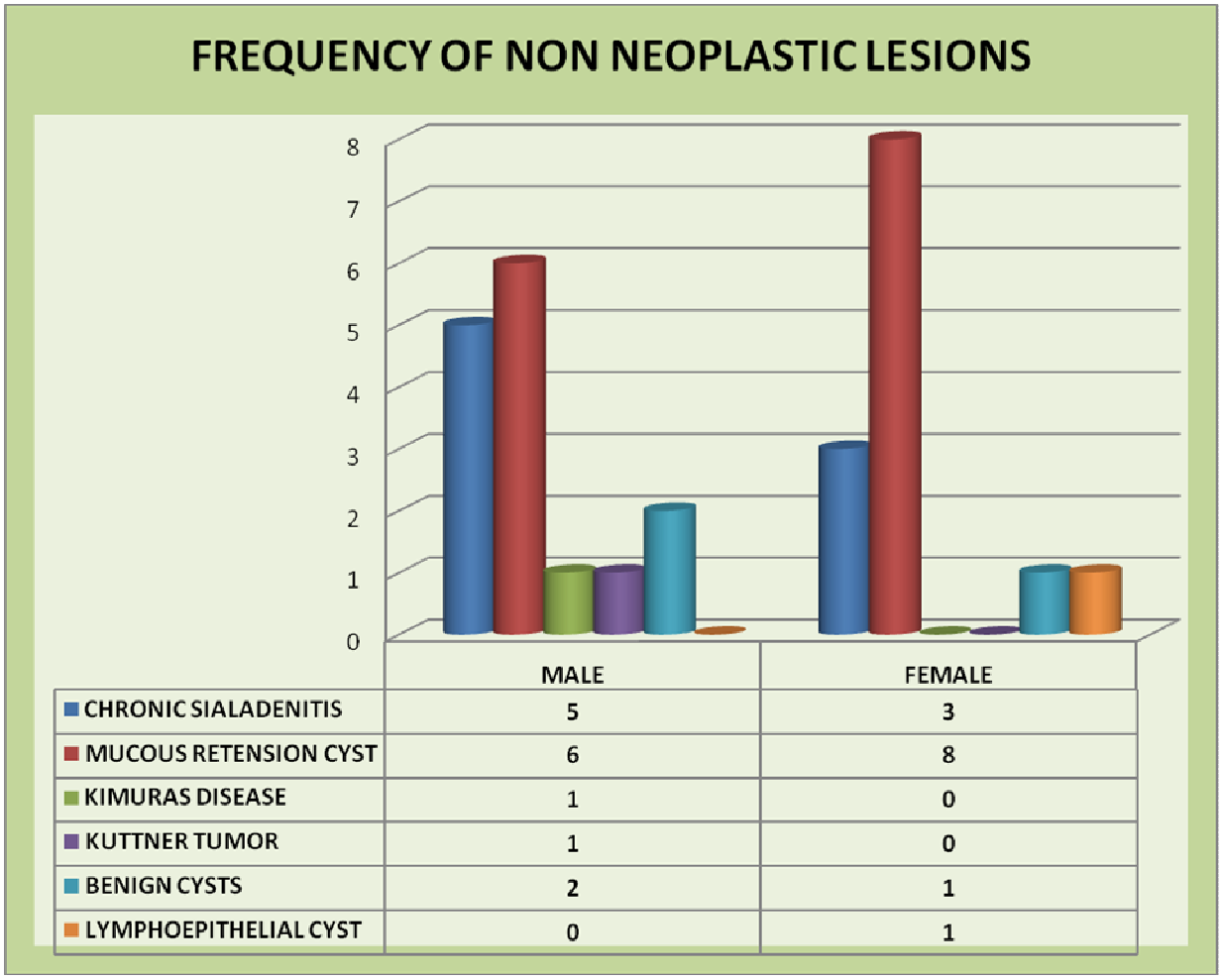


CHART 10



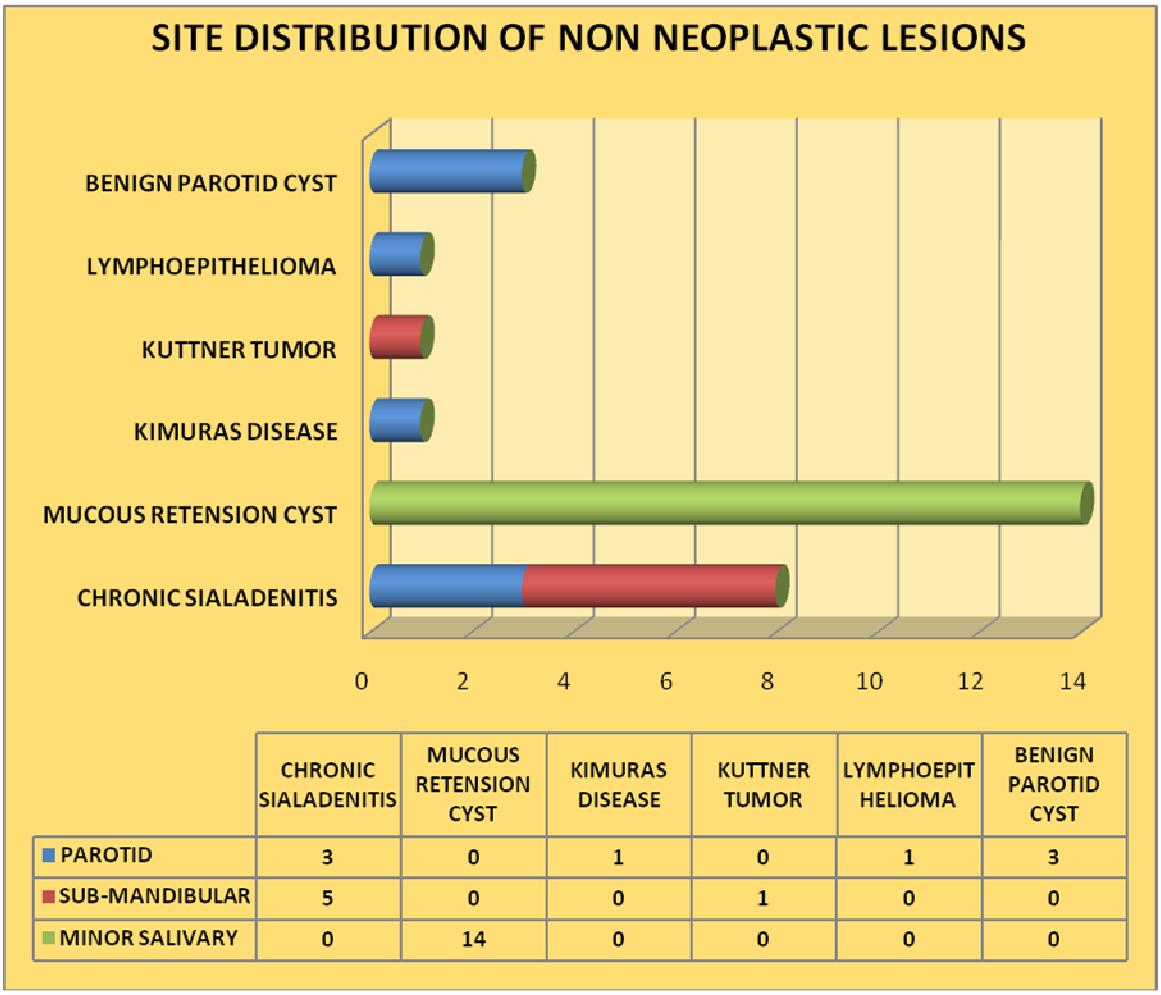
	MUCOEPIDERMOID CARCINOMA	ADENOID CYSTIC CARCINOMA	CARCINOMA EX PLEOM.ADENOMA	SALIVARY DUCT CARCINOMA	BASAL CELL ADENOCARCINOMA	PRIMARY SQUAMOUS CARCINOMA
PAROTID	12	1	1	0	0	1
SUB-MANDIBULAR	0	2	0	1	0	0
MINOR SALIVARY GLAND	0	2	1	1	1	0

CHART 11



	MALE	FEMALE
CHRONIC SIALADENITIS	5	3
MUCOUS RETENSION CYST	6	8
KIMURAS DISEASE	1	0
KUTTNER TUMOR	1	0
BENIGN CYSTS	2	1
LYMPHOEPITHELIAL CYST	0	1

CHART 12



	CHRONIC SIALADENITIS	MUCOUS RETENSION CYST	KIMURAS DISEASE	KUTTNER TUMOR	LYMPHOEPIT HELIOMA	BENIGN PAROTID CYST
PAROTID	3	0	1	0	1	3
SUB-MANDIBULAR	5	0	0	1	0	0
MINOR SALIVARY	0	14	0	0	0	0

CHART 13

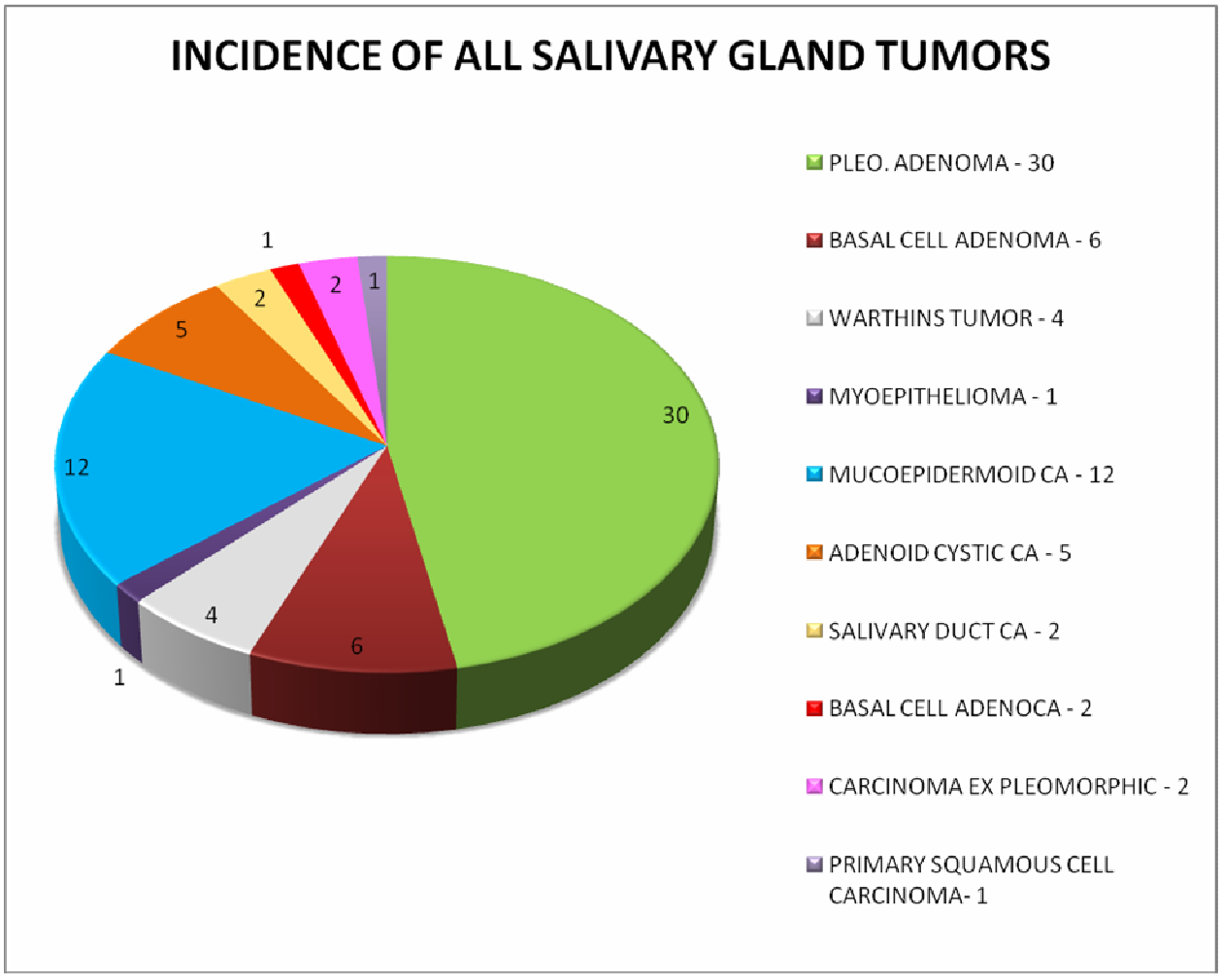

INCIDENCE OF ALL SALIVARY GLAND TUMORS
30
6
4
1
12
5
2
1
2
1
PLEO. ADENOMA - 30
BASAL CELL ADENOMA - 6
WARTHINS TUMOR - 4
MYOEPITHELIOMA - 1
MUCOEPIDERMOID CA - 12
ADENOID CYSTIC CA - 5
SALIVARY DUCT CA - 2
BASAL CELL ADENOCA - 2
CARCINOMA EX PLEOMORPHIC - 2
PRIMARY SQUAMOUS CELL CARCINOMA- 1

CHART 14

INCIDENCE PER YEAR OF SALIVARY GLAND TUMORS IN DIIFFERENT SERIES.

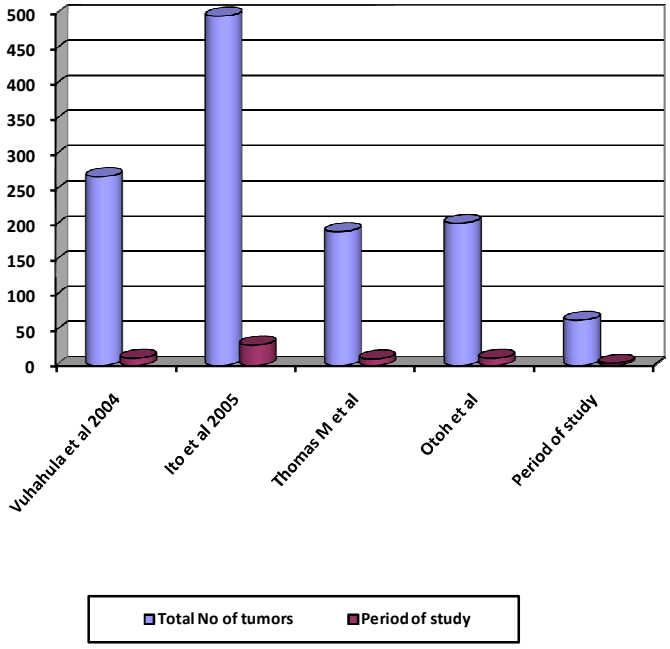


CHART 15

FREQUENCY OF BENIGN AND MALIGNANT TUMORS.

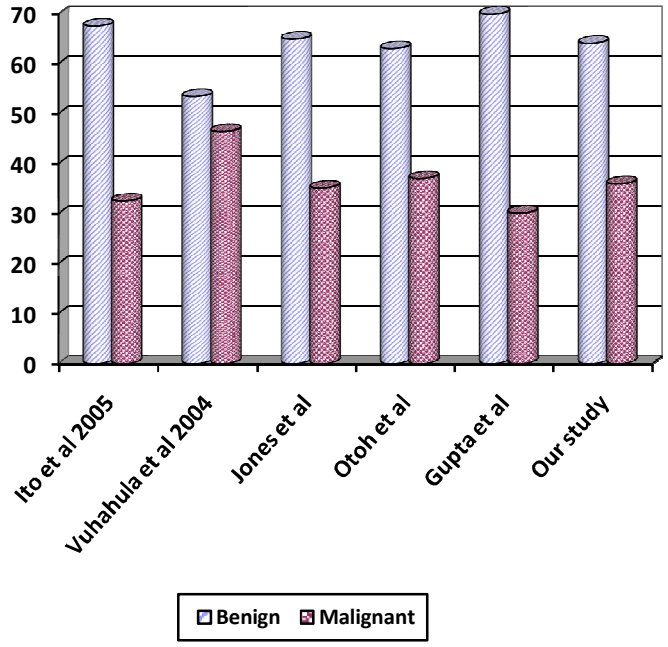


CHART 16

AGE DISTRIBUTION IN DIFFERENT SERIES.

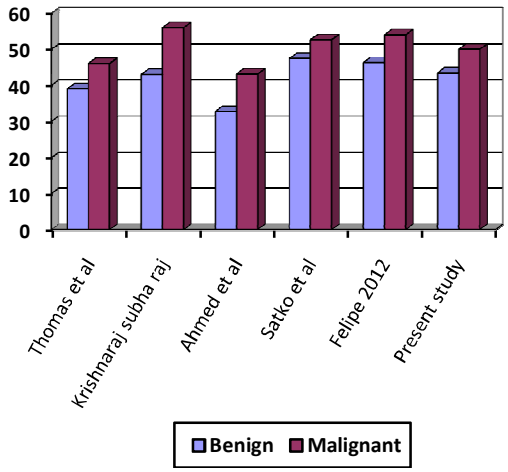


CHART 17

SITE DISTRIBUTION OF SALIVARY GLAND TUMOURS

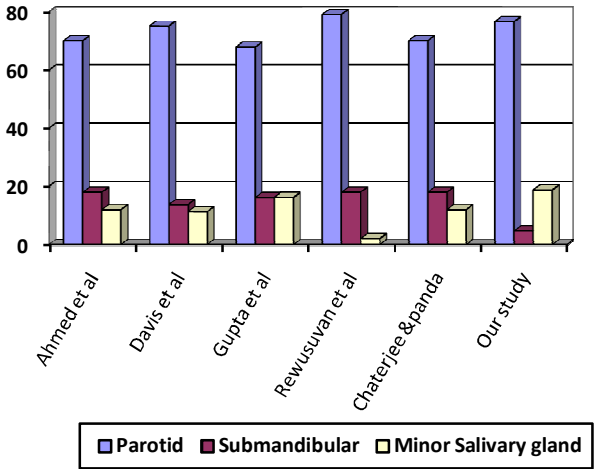


CHART 18

INCIDENCE OF PLEOMORPHIC ADENOMA IN VARIOUS STUDIES

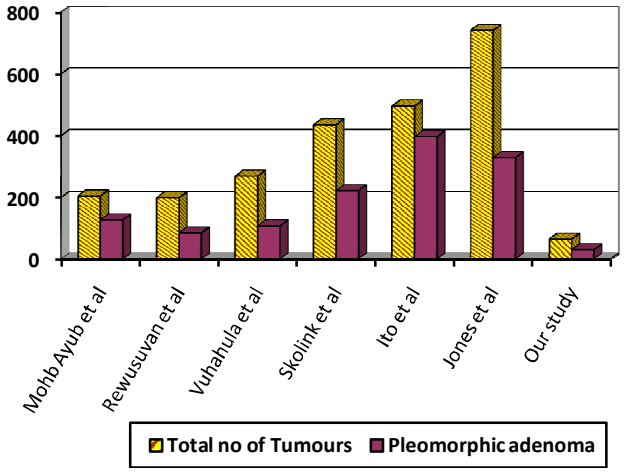


CHART 19

SITE DISTRIBUTION OF PLEOMORPHIC ADENOMA IN VARIOUS STUDIES

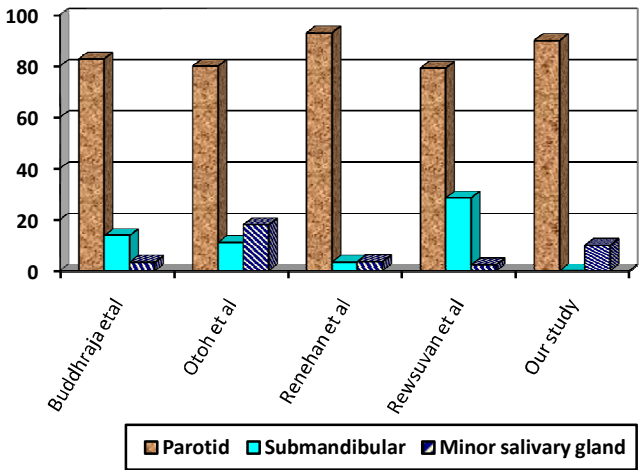


CHART 20

INCIDENCE OF BASAL CELL ADENOMA.

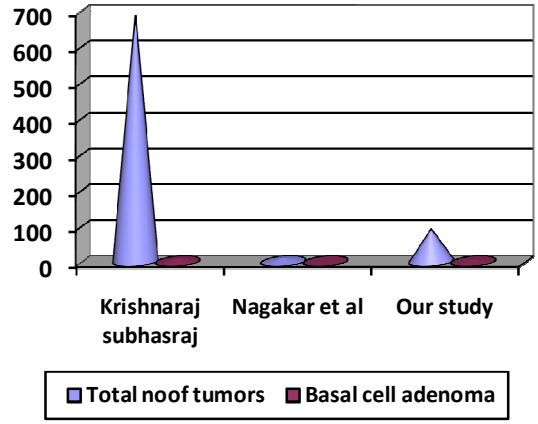


CHART 21

INCIDENCE OF WARTHINS TUMORS IN VARIOUS:

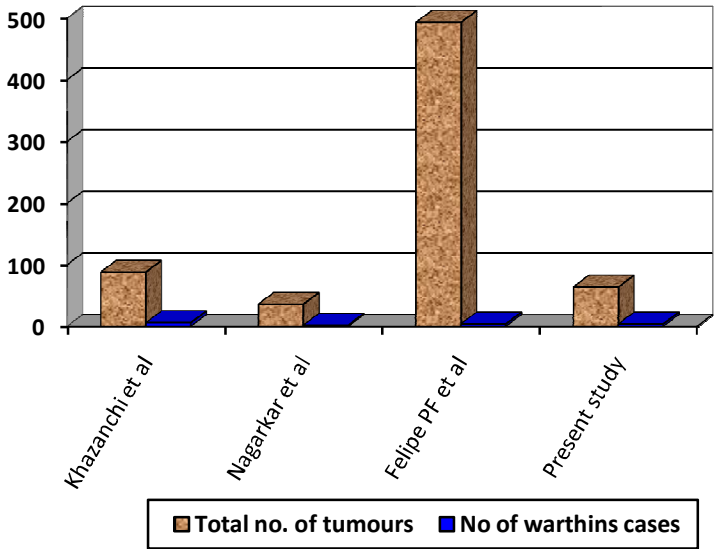


CHART 22

LOCATON OF WARTHINS TUMOR IN VARIOUS STUDIES;

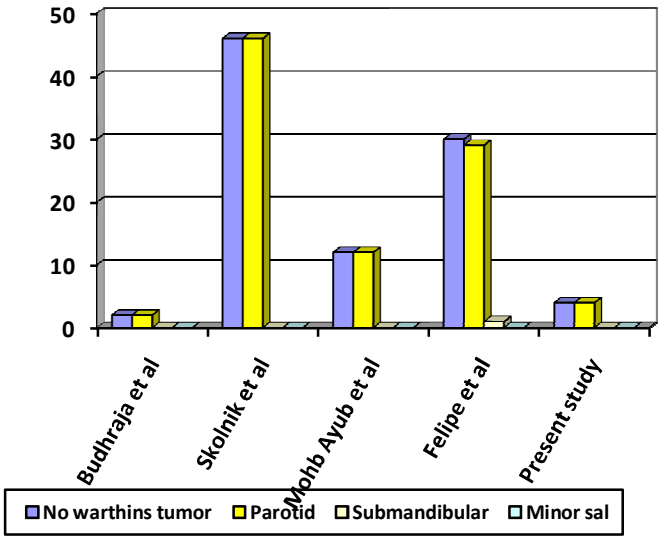


CHART 23

INCIDENCE OF MUCOEPIDERMOID CARCINOMA IN VARIOUS STUDIES:

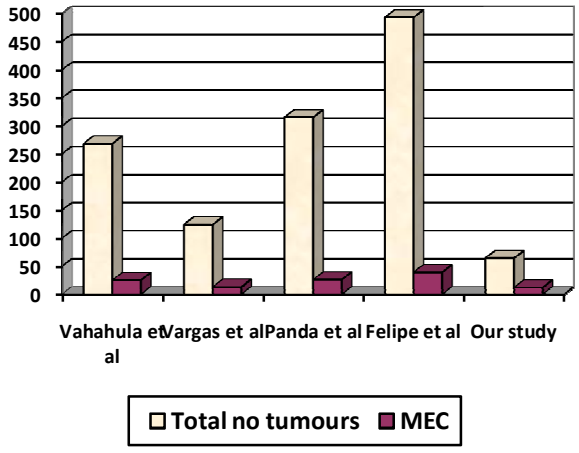


CHART 24

HISTOLOGICAL GRADING OF MUCOEPIDERMOID CARCINOMA.